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14. ABSTRACT This project is focused on novel tumor vaccines directed at MUC1 and other tumor antigens. Our specific aims are: 1)To assess the effectiveness of vaccines against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice; 2)To translate an effective vaccine strategy into a phase I clinical trial in patients with undetectable disease following standard therapy. The model of spontaneous mammary cancer is the MUC1-expressing polyoma middle T antigen mice (MMT). We have tested five vaccines in the preclinical mouse model and all elicited a strong immune response. The vaccine using MUC1 class I binding peptides prevented MUC1-expressing tumor growth. We have designed the Phase I clinical trial using a peptide vaccine comprised of MUC1 and HER-2/neu MHC class I peptides and HER-2/neu MHC class II peptide with unmethylated CpG oligodeoxynucleotides and GM-CSF as adjuvants in breast cancer patients free of disease. The clinical trial was unanimously approved by the Mayo Institutional Review Board (IRB 582-05) following receipt of FDA approval (BB-IND 12155) and by the DoD HSRRB in January 2007. Following receipt of the approvals, Pfizer agreed to supply the CpG7909(PF-3512676) for the clinical trial, as Pfizer has licensed the CpG from Coley Pharmaceuticals. Amended documents showing the change in supplier of CpG were submitted to the DoD HSRRB for final approval and to the FDA. Final approval from the DoD HRPO was received June 9, 2008. The clinical trial opened August 28, 2008. Two patients have been registered and 16 patients are in pre-registration.				
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INTRODUCTION

This project is focused on the development of novel tumor vaccines directed at MUC1, a transmembrane mucin that is aberrantly expressed in cancer. MUC1 is expressed on greater than 90% of breast cancers and often elicits cellular and humoral immune responses in humans. However, these responses are not sufficiently strong to eradicate tumors. MUC1 is a candidate peptide for novel immunotherapy strategies to strongly activate the immune system to eradicate tumors expressing these epitopes. In tumors, there is strong over expression of MUC1 on tumor cells and in circulation, expression is no longer restricted to the apical domain of cells, and glycosylation is altered, revealing immunodominant tumor-specific peptide sequences.

In our preclinical studies we have utilized mice that develop spontaneous mammary gland cancer that express MUC1. MUC1 transgenic mice (MUC1.Tg) were bred with mice carrying the MMTV-driven polyoma middle T antigen (MT) to create MMT mice. Mice transgenic for this protein develop B and T cell tolerance and are refractory to immunization with the protein encoded by the transgene. All mice are congenic on the C57BL/6 background to eliminate strain-specific modifier effects. In the MMT mice, mammary gland tumors are induced by the action of a potent tyrosine kinase activity associated with the polyoma virus middle T antigen driven by the mouse mammary tumor virus long terminal repeat (MMTV) [2]. Middle T specifically associates with and activates the tyrosine kinase activity of a number of c-src family members, eliciting tumors when a threshold level of gene product has been attained. This promoter is transcriptionally active throughout all stages of mammary gland development and results in widespread transformation of the mammary epithelium and the rapid production of multifocal mammary adenocarcinomas in 100% of the female mice. The MMT mouse appears to be an appropriate model for human cancer and allows us to study the effects of self-tolerance, immunity and auto-immunity to MUC1 as mammary tumors develop spontaneously.

The **hypothesis** of our study is that enhancing MUC1-specific immunity will result in anti-tumor immunity. We propose to develop an optimal cancer vaccine using epithelial cell mucin MUC1 peptides or protein or MUC1-expressing tumors presented by DCs as immunogen. The most successful therapies will be tested in a phase I clinical trial. An additional hypothesis is that tolerance occurs within the tumor environment, although immunization strategies can be developed to overcome tolerance.

RESULTS (BODY)

Specific Aims:

Specific Aim 1: To assess the effectiveness of vaccine formulations against MUC1 and other tumor antigens in the prevention and treatment of spontaneous breast carcinomas in mice.

Our preclinical studies were completed at the end of year three and the papers describing the results were included in the progress report for 2006.

Specific Aim 2: To translate the most effective vaccine strategies into phase I clinical trials in patients with high and low tumor burden.

Section II - Description of Regulatory Status of the Clinical Trial

The clinical trial opened August 28, 2008, at all three Mayo Clinic sites. On October 10, 2008, 2 patients have been registered and 16 patients are in pre-registration. Pre-registration involves determination of HLA-A2 status (must be positive) and MUC1 positivity on immunohistochemistry of invasive carcinoma. Following these analyses, the patient undergoes a physical exam and blood work.

Patient Population

Breast cancer is the most common type of tumor seen at Mayo Clinic. Considering all three sites, a total of 1,736 new breast cancer patients were seen in 2003 including 1,084 at the Rochester campus, 228 at the Jacksonville campus and 424 at the Scottsdale campus. We have access to all the patients with breast cancer seen at all three Mayo campuses. In addition to a large clinical practice, the Mayo Clinic records system allows review of patient data going back almost one century. We also have data from breast cancer

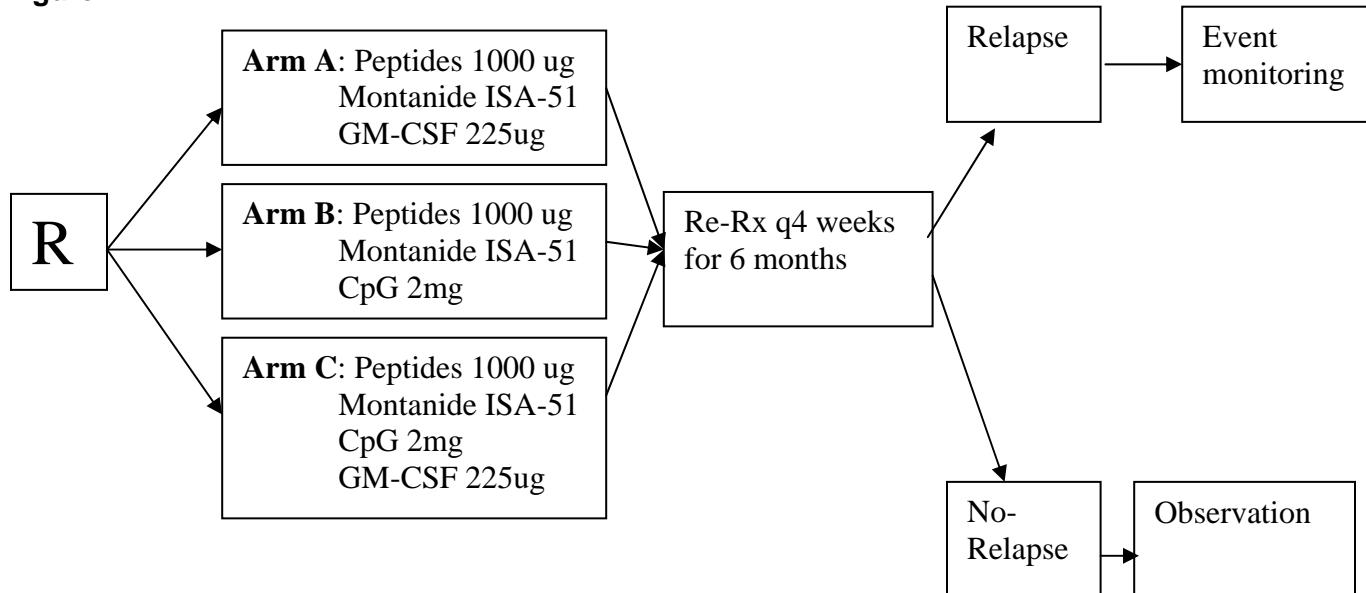
patients entered on prospective clinical trials over the last 30 years. Since 2000 we have prospectively recruited 877 women to a breast cancer patient registry in which a lifestyle and family history questionnaire is obtained. Of these 877 patients, 804 (92%) have provided a blood sample for DNA extraction and 364 patients (42%) have paraffin-embedded tissue available. We have compiled a list of patients that would be eligible for this clinical trial. Once we have completed the review process, these patients will be contacted regarding their interest in participating in the trial. Interest is very high, as immunotherapy has been shown to be a non-toxic therapy.

The trial will test MUC1 class I peptide (STAPPVHNV), HER-2/neu class I peptide (ILHNGAYSL) and HER-2/neu class II peptide (KVPIKWMALESIL) (1000 μ g of each peptide) delivered in Montanide ISA-51 and compare GM-CSF with unmethylated CpG oligodeoxynucleotides (PF 3512676) as immune adjuvants. Few vaccines have been tested in the optimal setting of minimal residual disease. CpG unmethylated oligodeoxynucleotides are a novel adjuvant that promote strong, antigen-specific T cell responses and help to overcome immune tolerance.

The peptides have been synthesized and vialled by Clinalfa (Merck Biosciences AG) and are being stored at the Mayo Clinic Comprehensive Cancer Center in Rochester. The peptides are certified for use in human studies and they fully comply with the international ethical and scientific quality standards, as stated in "ICH Guideline E6: Good Clinical Practice". The "Certificate for Analysis" for each peptide was included in the annual report for the last year.

The schema for the clinical trial is shown (Fig. 1).

Figure 1



KEY RESEARCH ACCOMPLISHMENTS

- The preclinical research was completed and described in the annual reports for years 3 and 4.

REPORTABLE OUTCOMES

- The Clinical Trial was activated on August 28, 2008, at Mayo Clinic Arizona, Mayo Clinic Rochester, Minnesota and Mayo Clinic Jacksonville, Florida. The activation memo is included in the Appendix.

Time Table of Protocol Development

- Clinical protocol concept approved by Mayo Clinic Cancer Center 12-11-03
- Completed Mayo Clinic Cancer Center Peer Review process 5-4-04
- List of recommendations by FDA (pre IND conference) 4-21-04
- Peptides synthesized and vialled by ClinAlfa® for use in this clinical trial:
 1. Her-2/neu (435-443)
 2. Her-2/neu (883-899)
 3. MUC1 (950-958)
- Completion of IND documentation and submission to FDA on December 17, 2004.
- FDA approval (IND # 12155)
- Mayo IRB approval April 22, 2005 (IRB 782-05)
- Submission to DOD HSRRB on May 11, 2005
- Submission to FDA of the revised 1572 and Investigator's Brochure on September 15, 2005
- Submission to Mayo IRB of amendment, which excludes prisoners from the study population and reduces the number of personnel involved in the study (September 12, 2005)
- Submission of revision to HSRRB on February 10, 2006 (response to request for revisions from 14 December 2005 HSRRB meeting)
- Submission of revision to HSRRB on May 18, 2006
- Submission of final documents to the Mayo IRB August 30, 2006
- Final approval Mayo IRB December 15, 2006
- Final approval DoD HSRRB (Log Number A-10856) January 26, 2007
- CpG-7909 adjuvant to be supplied by Pfizer as PF3512676 for this clinical trial.
- Submission of revised clinical protocol to Mayo IRB, HSRRB and IND (July, August 2007)
- Final Mayo IRB approval August 2, 2007
- Extension of "performance period" by 24 months to 14 September 2009

- Approval from DOD HRPO on June 9, 2008
- Clinical Trial was activated August 28, 2008.

CONCLUSIONS

The past year has been spent revising the clinical protocol and patient consent and receiving approval from the HSRRB. Following approval from all regulatory boards, we found that CpG supply would have to come from Pfizer, Inc rather than from Coley Pharmaceuticals because of Coley's licensing of the rights for use of CpG in cancer vaccines to Pfizer. Protocols were modified accordingly after Pfizer agreed to supply the CpG (now called PF 3512676). Final Mayo IRB approval to the revised clinical protocol was given August 2, 2007. Final approval from the DOD HRPO was received June 9, 2008. The trial opened August 28, 2008.

APPENDICES

The appendix includes the activation notification and other MC0339 clinical trial documents.

A – Activation notification

B – MC0338 clinical trial protocol “MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas”

C – Forms Packet

D – Pre-registration Eligibility Checklist

E – Registration (Step 2) Eligibility Checklist

F – Revised Activation Memo

G – Consent Form – Markovic: Mayo Clinic Rochester

H – Consent Form: Perez, Mayo Clinic Jacksonville

I – Consent Form: Pockaj, Mayo Clinic Arizona

J – Amendment Approval Memo from the DOD

Memo

Date: August 29, 2008

To: MC0338 Study Team

From: Jane Milburn
Protocol Development Coordinator

Re: **MC0338; MUC1/HER-2/neu Peptide-Based Immunotherapeutic Vaccines for Breast Adenocarcinomas**

Study Chair: Dr. Markovic

The above study is now activated for patient enrollment.

A protocol and forms packet are enclosed. Please note the following information regarding the pre-registration component.

- Submit 1 H&E and 6 unstained slides to Dr. Sandra Gendler in Mayo, AZ (c/o Cathy Madsen at the following address) by FedEx and a tracking number sent to Sandra Gendler and Cathy Madsen.
Cathy S. Madsen
Senior Research Technologist for Dr. Sandra Gendler
Mayo Clinic Scottsdale
Johnson Research Building SCJ 2-221
13400 E. Shea Blvd.
Scottsdale AZ 85259
- The slides from patients enrolled at MCR will be sent by Christie Maszk (Pathology Coordinator). The Central Testing of MUC1 Expression Form will be filled out (top portion) and scanned and sent by e-mail to Dr. Ann McCullough (McCullough.ann@mayo.edu), Sandra Gendler (Gendler.sandra@mayo.edu), Cathy Madsen (cathy.madsen@mayo.edu) and Jennifer Roedig (roedig.jennifer@mayo.edu).
- The slides from patients enrolled at MCS will be sent by Leslie Dixon to Cathy Madsen. The Central Testing of MUC1 Expression Form will be filled out (top portion) by Jennifer Roedig and scanned and sent by e-mail to Dr. Ann McCullough (McCullough.ann@mayo.edu), Sandra Gendler (Gendler.sandra@mayo.edu), Cathy Madsen (cathy.madsen@mayo.edu) and Jennifer Roedig (roedig.jennifer@mayo.edu).
- The slides will be stained in Dr. Gendler's lab.
- Cathy will log and forward the slides to Dr. Ann McCullough in Scottsdale for central pre registration review of MUC1 expression.
- The completed Central Testing of MUC1 Expression Form will be faxed to the Registration Office at 4-0885 and to the Pathology Coordinator, Christine Maszk, at 4-9628 by Eleanor Gray of Dr. McCullough's lab as soon as possible after test results become available.
- Slides and correlating paperwork will be returned to Cathy Madsen for Dr. Gendler's lab.

If you have any questions, please feel free to contact me directly.

Mayo Clinic Cancer Center

**MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines
for Breast Adenocarcinomas**

Principal Investigators/Study Chairs: Svetomir Markovic, M.D., Ph.D. *⁺
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Study Co-chairs: James N. Ingle, M.D (Mayo Clinic, Rochester)
Pinky Mukherjee, Ph. D (Mayo Clinic, Scottsdale) ✓
Tom Fitch, M.D (Mayo Clinic, Scottsdale)
Barbara Pockaj, M.D (Mayo Clinic, Scottsdale)
Edith A. Perez, M.D. (Mayo Clinic, Jacksonville)

Statistician: Vera J. Suman, Ph.D. ✓

* Investigator having primary responsibility for this protocol

⁺ IND sponsor (IND# 12155)

✓ Study contributor(s) not responsible for patient care.

<u>Document History</u>	<u>(effective date)</u>
IRB approved draft version:	April 22, 2005
Revised draft version:	February 3, 2006
Revised draft version	July 25, 2007
Revised draft version	January 18, 2008
Revised draft version	April 23, 2008
Activation	August 28, 2008

Protocol Resources

Questions:	Contact Name:
Review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event	Robert R. McWilliams, M.D. Medical Monitor Phone: 507-284-8432 E-mail: mcwilliams.robert@mayo.edu
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion	Carol Leonard Quality Control Specialist Phone: 507-284-3121 Fax: 507-284-1902 E-mail: leonard@mayo.edu
Drug administration, infusion pumps, nursing guidelines	Lisa Kottschade, NP Mayo Clinic Cancer Center Nurse Phone: 507-538-2958 E-mail: kottschade.lisa@mayo.edu
Clinical data submission and record maintenance	Renee Bradshaw Clinic Research Associate Phone: 507-284-2041 E-mail: Bradshaw.renee@mayo.edu
Protocol document, consent form, Regulatory issues	Jane M. Milburn, MBA Protocol Development Coordinator Phone: 507-266-0743 Fax: 507-284-5280 E-mail: milburn@mayo.edu
Technical problems with electronic form entry	Vicki Bryhn Data Management Specialist Phone: 507-266-5350 Fax: 507-538-0906 E-mail: bryhn@mayo.edu

Index**Schema**

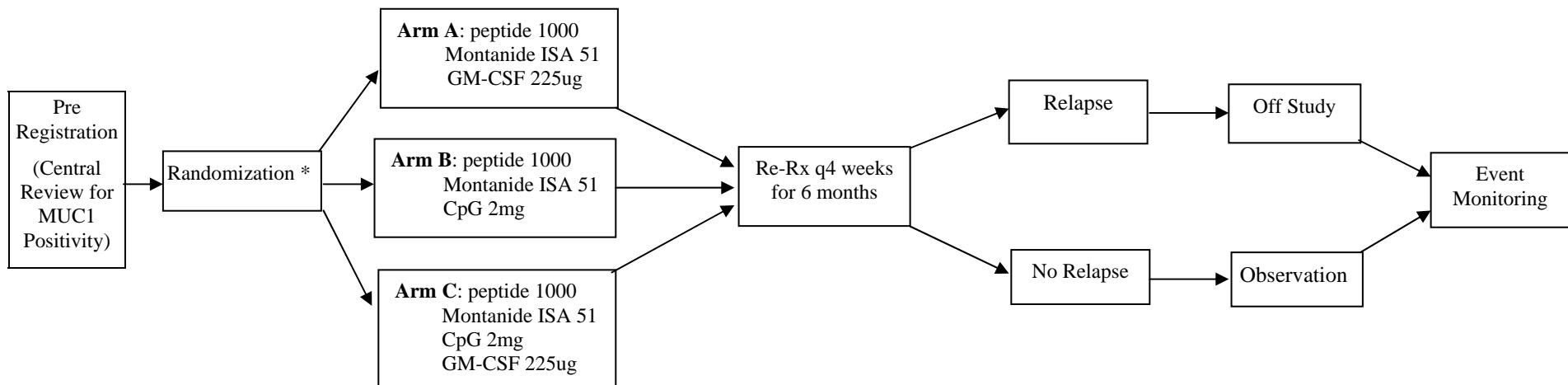
- 1.0 Background
- 2.0 Goals
- 3.0 Patient Eligibility
- 4.0 Test Schedule
- 5.0 Grouping Factors
- 6.0 Registration/Randomization Procedures
- 7.0 Protocol Treatment
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- 9.0 Ancillary Treatment/Supportive Care
- 10.0 Adverse Event (AE) Reporting and Monitoring
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- 19.0 Budget Considerations
- 20.0 References

Appendix I ECOG Performance Status Scale

Appendix II Injection Site Record

Consent Form

Schema



* Patient must have MUC1 positive breast cancer confirmed by central review prior to randomization.

Generic Name	Brand Name	Mayo Abbreviation	Availability
MUC1 (STAPPVHNV)		MUC1	Clinalfa
HER2 Peptide-1 (ILHNGAYSL)		HER2-1	Clinalfa
HER2 Peptide 2 (KVPIKWMALESILRRRF)		HER2-2	Clinalfa
Montanide ISA 51		MONTAN	Purchased from Seppic
GM-CSF (Sargramostim)	Leukine	GM-CSF	Commercial
CpG7909 (PF-3512676)		CP7909	Pfizer Pharm, Inc.

1.0 Background

1.1 Breast cancer is diagnosed in 200,000 individuals in the United States annually and contributes to approximately 40,000 deaths each year. For tumors confined to the breast, surgical removal provides a good prognosis. However, primary tumor that metastasizes to distant sites, such as lymph nodes, lungs, liver and brain, correlates with a poor prognosis. Patients with advanced stage breast cancer are at high risk of relapse. Complications from metastatic disease are the leading causes of cancer-related deaths. Novel adjuvant strategies, such as breast cancer specific vaccines, are being considered as a clinical intervention that may reduce the chance of recurrence.

In recent years there has been great interest in the development of these cancer vaccines, which are designed to immunize individuals to antigens present on tumors. Cancer vaccines are a non-toxic therapy, which have been shown in several melanoma trials to have the potential of controlling disease and prolonging survival because tumors can be surgically removed and there is often a long period of time before the tumor recurs at metastatic sites, cancer vaccines have been proposed as an optimal therapy that could prolong the time to recurrence. This optimal opportunity of immunization in the situation of minimal residual disease has rarely been tested, however, as most vaccines have been given to patients with large tumor burden after the failure of standard therapies in Phase I and Phase II trials. Recently, several groups have addressed the use of adjuvant immunotherapy following complete surgical resection [1]. Data from these studies are not yet complete.

1.2 The past two decades in tumor immunology have led to the discovery of specific tumor antigens that have been shown in preclinical studies to elicit tumor-specific immunity and establish long term memory without autoimmunity. For breast cancer, vaccines composed of epitopes derived of MUC1, HER-2/neu, MAGE3, CEA have been studied and shown to be immunogenic without causing autoimmunity [2-5].

1.3 It is now clear that tumor antigens are presented in the context of specific class I and Class II HLA molecules. Class I presentation, in the presence of appropriate co-stimulation, is thought to stimulate a cytolytic CD8⁺ T cell response, while antigen presentation in the context of Class II molecules stimulates a CD4⁺ helper T cell response [6].

1.4 One approach for the development of a cancer vaccine is the use of tumor associated synthetic antigens for immunologic priming. Because specific peptides are ubiquitous in tumors of the same histologic type, identical peptide vaccines may be employed in allogeneic hosts bearing the same tumor histology. Additionally, the use of single peptides for immunization limits the potential induction of undesired autoimmunity [7-9]. Recent developments in the use of soluble MHC Class I/peptide tetramers and elispot technology have enabled rapid characterization of epitope-specific CTL responses [10, 11]. In addition to being well-explored and understood, many of these antigens are shared tumor antigens. Vaccines that are composed of these antigens can be developed for use in a large number of patients. The primary limitations to peptide based vaccine strategies are haplotype restriction, potential for degradation, and uncertainty regarding which peptides, used alone or in combination, are the most immunogenic [12, 13]. This study is designed to test these uncertainties.

1.5 One attractive and broadly applicable target for immunotherapeutic strategies is the MUC1 tumor antigen. MUC1, a cell-associated mucin, is expressed on the cell surface of many epithelial malignancies as well as by hematological malignancies [14-17]. These include multiple myeloma (92%) and acute myelogenous leukemia (67%) [18]. Greater than 90% of breast carcinomas express MUC1; high levels are also found in adenocarcinomas originating from most tissues [14, 16]. MUC1 expression is greatly up-regulated on tumors (reviewed in

Gendler [19]). Expression on tumors is no longer apical, but it is found all around the cell surface and in the cytoplasm. In addition, glycosylation on tumor-synthesized MUC1 is aberrant, with greater exposure of the peptide core than is found in normal tissues. MUC1 has long been an interesting target molecule for immunotherapeutic strategies, given its high level and ubiquitous expression. Patients with tumors, especially with breast, pancreas and ovarian tumors, have exhibited immune responses to MUC1 with the presence of antibodies and T cells specific for MUC1 detected in about 10% of individuals. An HLA unrestricted T cell response among cancer patients has also been described [20-23]. There is increasing evidence from murine and human studies that MHC-restricted T cells can be induced in mice and humans after immunization with the MUC1 peptide or MUC1 antigenic epitopes [24-32]. Importantly, there have been reports of two HLA-A2 binding peptides derived from the MUC1 protein [33]. One of the peptides is from the tandem repeat sequence of MUC1 and the second peptide is from the signal sequence. MUC1-specific cytotoxic T cells (CTLs) have been induced in T cells from healthy donors following *in vitro* immunization using peptide-pulsed dendritic cells. MUC1-specific CTLs have also been induced *in vivo* after vaccination of breast and ovarian cancer patients with peptide-pulsed DCs [18].

1.6 A second candidate for peptide-based immunotherapy is HER-2/neu, the gene product of the erbB2/neu protooncogene. HER-2/neu is overexpressed in approximately 30% of breast cancer patients. HER-2/neu is also expressed by multiple types of tumors, including ovarian, lung, colon, pancreas and gastric tumors [34-36]. HER-2/neu has particular relevance, as it is expressed at high levels in early *in situ* lesions in breast carcinoma [37]. Thus, it is a target for early disease. Immunologic responses to HER-2/neu have been detected in a minority of patients with advanced stage breast and ovarian cancer, including antibodies, T helper and CD8 responses [38, 39]. Several HLA-class I binding peptides have been previously identified. A novel HLA-A2.1 binding peptide from the HER-2/neu extracellular domain [HER-2(9₄₃₅)] was recently identified [40]. This peptide (ILHNGAYSL) bound to HLA-A2.1 with intermediate affinity (IC50 74.6 nM). The HER-2(9₄₃₅) epitope was tested using an *in vitro* immunization protocol and found to elicit CTLs that killed peptide-sensitized target cells. The CTLs elicited also recognized the HER-2/neu antigens, as it specifically killed tumor cells expressing the HLA-A2.1 and HER-2/neu antigens (see below in preliminary data). Furthermore, recognition of the tumor cell targets was significantly inhibited by unlabeled (cold) targets pulsed with HER-2(9₄₃₅), but not by unlabeled targets either unpulsed or pulsed with a control HLA-A2.1 binding peptide (see below). Thus, the CTLs induced by HER-2(9₄₃₅) are antigen specific.

A potential limiting factor for peptide based immunotherapy is related to a defined antigenic repertoire which is HLA restricted. This factor, inherent to all peptide-based approaches, restricts patient access. Additionally, because individual peptides only have the potential to induce epitope-specific CTL, the vast majority of potential tumor antigens are not targeted. In this setting, tumor down regulation of individual antigens or HLA epitopes promotes immune evasion. Recent evidence, however, suggests that this problem of epitope restriction may not be as physiologically important as was previously postulated. Specifically, it has now been clearly demonstrated that a T cell response induced against one epitope can stimulate CTL response to other target epitopes through a mechanism termed epitope spreading [3, 41, 42]. Using an experimental autoimmune encephalitis model, Vanderlugt et al. have demonstrated that disease progression is associated with the development of epitope-specific helper T cells, which are distinct from those initiating the disease. Transfer of secondary CD4⁺ cells to naïve mice induces the disease phenotype and the disease is abrogated by blocking the secondary T cell response even though the primary T cell response remains intact [43, 44]. Disis demonstrated epitope spreading in 84% of patients vaccinated with HER-2/neu peptides, reflecting the initiation of an endogenous immune response. The immunity persisted after active immunizations ended [3]. These data suggest that peptide based approaches to cancer immunotherapy may indirectly stimulate multiple tumor reactive CTL against minor antigens in

the presence of residual tumor. Based on this concept, the current study is designed as a therapeutic approach, with peptide epitope selection designed to enhance the number of potential candidates.

In addition to class I epitopes, immunogenic HLA-DR restricted class II epitopes have been defined for HER-2/neu. CD4⁺ helper T lymphocytes (T_H) responses play an essential role in immunologically mediated anti-tumor immunity [45]. T_H lymphocytes provide CTLs with growth-stimulating cytokines, prime/activate DCs to effectively present antigen to naive CTL precursors [46-48] and they are important in the development of immune memory [49-51]. The development of IgG antibodies to HER-2/neu and the identification of CD4⁺ T cells that secrete cytokines in response to HER-2/neu peptides or recombinant HER-2/neu protein suggest responses to helper T cells [52-57]. A promiscuous MHC class II T_H epitope has been identified for the HER-2/neu antigen (HER-2₈₈₃). T cell responses are restricted by HLA-DR1, HLA-DR4, HLA-DR52, and HLA-DR53 [58]. Peptide-induced T cells were effective in recognizing naturally processed HER-2/neu protein. The peptide HER-2₈₈₃, (KVPIKWMALESILRRRF), which was selected by computer algorithm, was tested for its capacity to stimulate CD4⁺ T cells isolated from four healthy, MHC-typed individuals (DR1/11, DR1/13, DR4/15, DR7/17) in primary *in vitro* culture using peptide pulsed autologous DCs. T cells that proliferated were found to react with peptide and recombinant HER-2/neu intracellular domain protein presented by autologous DCs (see below). These results, showing reactivity with recombinant protein, suggest that HER-2₈₈₃ is naturally processed, as the peptide stimulated T cells react with DCs primed with recombinant protein. Clearly, HER-2₈₈₃ is a naturally processed peptide epitope and is promiscuous for multiple HLA-DR epitopes, making it an ideal candidate for therapeutic applications.

1.7 Because of the expression of MUC1 and HER-2/neu in multiple cancers, the development of this peptide-based immunotherapy can potentially impact the treatment of multiple disease entities, not only adenocarcinomas but hematopoietic malignancies as well. There is considerable interest in the use of the MUC1 peptide vaccination for treatment of multiple myeloma following transplant when there is minimal residual disease prior to remission.

1.8 GM-CSF

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a commercially available cytokine currently used in patients undergoing chemotherapy to shorten the duration of post-chemotherapy neutropenia. Recently published evidence also suggests that GM-CSF may play a role as an immune adjuvant [59, 60]. The following observations illustrate the mechanisms by which GM-CSF can potentiate the immunogenicity of an antigen: 1) GM-CSF is a key mediator of dendritic cell (DC) maturation and function [61]; 2) GM-CSF increases surface expression of class I and II MHC molecules as well as co-stimulatory molecules of dendritic cells *in vitro* [61]; 3) GM-CSF enhances antibody responses to known immunogens *in vivo* [62]; 4) tumor cells transfected with genes encoding/expressing GM-CSF are able to induce long lasting, specific anti-tumor immune responses *in vivo* [63]; 5) GM-CSF encapsulated in biodegradable microspheres mixed with whole tumor cells resulted in systemic anti-tumor immune responses comparable to those of GM-CSF transfected tumor cells [64]. Therefore, addition of GM-CSF to an oligopeptide antigen may substantially enhance its immunogenicity.

In an attempt to optimally enhance the immunogenicity of the peptides we will deliver the antigens and GM-CSF emulsified in incomplete Freund's adjuvant (IFA, Montanide ISA-51). This delivery mechanism should be comparable to a previously demonstrated delivery mechanism utilizing GM-CSF suspended in microspheres and mixed with tumor cells (antigens). We hypothesize that the emulsified GM-CSF in close proximity to tumor antigen peptides will substantially enhance their immunogenicity. This proximity of antigen and GM-CSF seems to be necessary for the adjuvant effect of GM-CSF, as systemic administration of

equivalent doses in animal models has not demonstrated adjuvant activity. Also, the adjuvant/local inflammatory properties of IFA may play a role in attracting antigen presenting cells to the site of injection [53]. We have preliminary data demonstrating the plausibility of such a mechanism.

1.9a Preliminary data demonstrating the feasibility of this approach already exists. Rosenberg and investigators published effective generation of peptide-specific T cells in melanoma patients immunized with peptides derived from gp100 [65]. Despite the demonstration of a specific immune response, no clinical responses were detected. Addition of systemic GM-CSF resulted in more pronounced CTL and delayed type hypersensitivity reactions and in a few cases objective tumor regressions. Salgaller et al. utilized a peptide derived from the gp100 epitope suspended in IFA and demonstrated generation of specific T cell responses to the peptide in melanoma patients [66]. Both studies suggest that increased immunogenicity of the peptide antigens leads to a more pronounced T cell response, which in some cases results in a clinically relevant anti-tumor effect. In the proposed study, we will combine the immunoadjuvant effects of both IFA and GM-CSF with the goal of increasing the immunogenicity of the MUC1 and HER-2/neu immunodominant peptides.

Preliminary observations in an ongoing clinical study (MC9973) utilizing HLA-A2 specific melanoma differentiation antigen peptide vaccines in which the peptide is emulsified in a suspension of IFA and GM-CSF is demonstrating enhanced skin reactions if peptide emulsified in IFA is administered in the presence of GM-CSF. A dose of 50 µg of GM-CSF in the presence of IFA and peptide results in extensive local skin reactions as well as evidence of a clinical response in one of seven patients thus far. No changes in the numbers of peptide specific CTLs were observed. However, a recent publication demonstrated superior numbers of vaccine specific CTLs generated in a peptide vaccine utilizing 225ug of GM-CSF in IFA) [67]. This would suggest a dose/response relationship of GM-CSF and anti-peptide vaccine CTL frequencies as determined by ELISPOT and tetramer assays. Therefore, in the current trial we propose to use 225ug of GM-CSF suspended in IFA (montanide ISA-51).

1.9b CpG

Therapeutic properties of bacteria in the treatment of malignant diseases (i.e. Coley's toxin) is an observation that has permeated the oncology literature for almost a century. More recently, it has been demonstrated that bacterial DNA possesses unique immunomodulatory features of potential utility in cancer therapy. Specifically, unmethylated CpG are able to stimulate NK cells and B cells. Furthermore, synthetic oligodeoxynucleotide (ODN) constructs containing unmethylated CpG motifs (CpG-ODN) were able to activate dendritic cells (DC) enhancing their antigen processing/presentation properties and stimulating production of Th1 cytokines necessary for CTL immune responses. Thus, CpG ODN appeared to function as an immune adjuvant. Several preclinical and clinical works illustrate the ability of CpG-ODN to function as a potent immune adjuvant for various forms of vaccines. One of the more interesting works, pertinent to this study, demonstrates the ability of CpG ODN to induce CTLs against a peptide vaccine when administered in conjunction with incomplete Freund's adjuvant (IFA) [68]. These authors used a MART-1/Melan-A₂₆₋₃₅ peptide emulsified in IFA with or without the addition of 50ug of CpG ODN to immunize human D^b (HHD) A2 transgenic mice. Their data suggest superior anti-peptide immunization in the CpG-ODN immunized group as determined by the frequency of tetramer positive CTLs. Our own data support these findings demonstrating superior immunization efficacy of IFA+CpG-ODN with ova peptide of C57BL/6 mice when compared to either IFA+peptide or complete Freund's adjuvant (CFA) + peptide (data not shown). An additional benefit to the CpG-ODN adjuvant is that it has been shown to be especially good at enhancing cellular and humoral immunity and promoting a Th1-type of response in older mice [69]. The population that develops cancer is mainly older individuals, thus the CpG-ODN adjuvant may be particularly relevant for this trial. Based on preclinical data

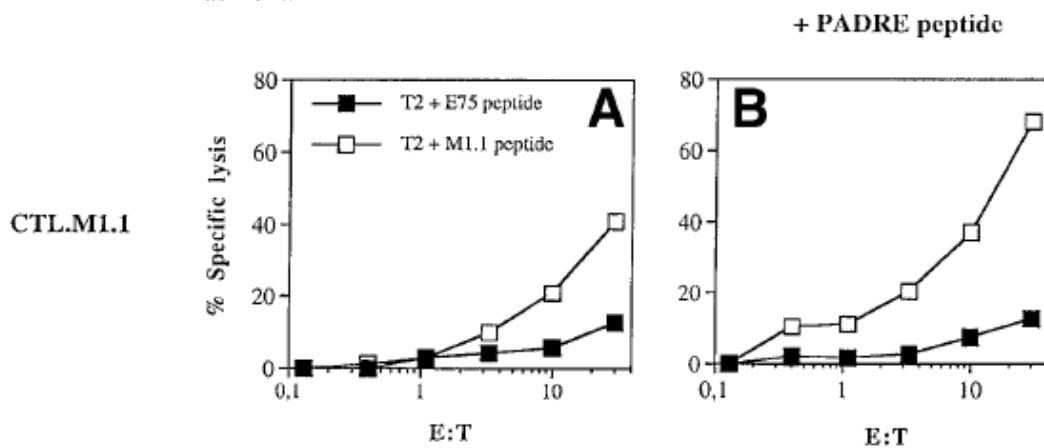
suggesting the potent immune adjuvant properties of CpG co-emulsified with peptides in IFA, we elected to test the efficacy of CpG-ODN in the setting of a peptide vaccine immunization in this clinical trial. The dose of CpG-ODN that we decided to use in this study is 2mg/vaccine. The dose is based on published data demonstrating a direct dose-dependent relationship of CpG-ODN (0.125 – 1.0 mg) and magnitude of measured immune responses (HepB vaccine adjuvant [70]). This is well below the highest tested doses of 20mg/week. Based on these observations we feel that the 2mg dose is a reasonable starting point for a CpG-ODN adjuvant suspended in Montanide ISA 51 alone or in combination with GM-CSF.

1.9c Preliminary Data

Preliminary data will be presented in multiple sections. First, we will provide data to support the choice of MUC1 and HER-2/erbB2 antigenic epitopes for this trial. Next, we will define our experience using peptides to stimulate tumor reactive T cells for cancer immunotherapy. Finally, we will discuss our experience with the immune adjuvants GM-CS and CpG-ODN. These preliminary data provide a strong foundation for the current proposal.

1.9c1 Identification of CTL Epitopes from MUC1

Using a computer analysis of the MUC1 amino acid sequence, two novel peptides were identified with a high binding probability to the HLA-A2 molecule [33]. Two peptides from MUC1 were identified; one from the tandem repeat M1.1 (STAPPVHNV₉₅₀₋₉₅₈) and one from the leader sequence M1.2 (LLLLTVLTV₁₂₋₂₀). The presence of the V in position 6 increases the binding of the M1.1 peptide to the HLA-A2 molecule. There is some variability in the tandem repeats in MUC1 and this sequence is found in the last tandem repeat. Cytotoxic T cells were induced from healthy donors by primary *in vitro* immunization using peptide-pulsed dendritic cells. The peptide-induced CTL lysed tumors endogenously expressing MUC1 in an antigen-specific and HLA-A2-restricted fashion..



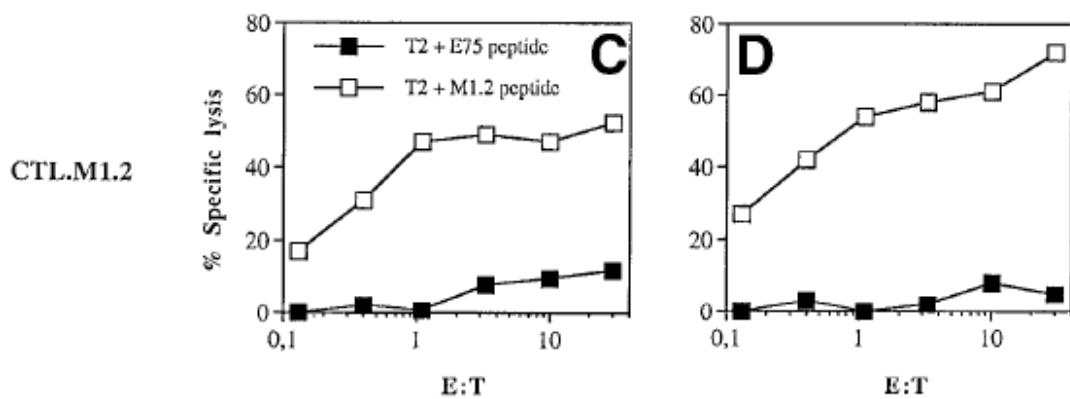


Figure 1. Induction of CTL responses by peptide-pulsed dendritic cells. Adherent peripheral blood mononuclear cells were grown for 7 days with GM-CSF, IL-4, and TNF alpha. DCs pulsed with the synthetic peptides derived from the MUC1 protein (M1.1 and M1.2) were used to induce a CTL response in vitro. In addition to the MUC1 peptide DCs were incubated with the PAN-DR binding peptide PADRE as a T-helper epitope. Cytotoxic activity of induced CTL was determined in a standard ^{51}Cr -release assay using T2 cells as targets pulsed for 2 hours with 50 μg of the cognate (open symbols) or irrelevant HER-2/neu protein-derived E75 peptide (solid symbols). (data reproduced from Brossart 1999 [33])

Next, the ability of the induced MUC1-specific CTL lines to lyse tumors expressing MUC1 was tested. MCF-7 cells that express MUC1 endogenously and are HLA-A2 positive were used as targets in a standard ^{51}Cr -release assay. The controls were SK-OV-3 cells, which express MUC1, but are HLA-A2 negative and the immortalized B cell line, Croft, which is A2 positive and was pulsed with MUC1 M1.1 or M1.2 peptides or the irrelevant HER-2/neu E75 peptide.

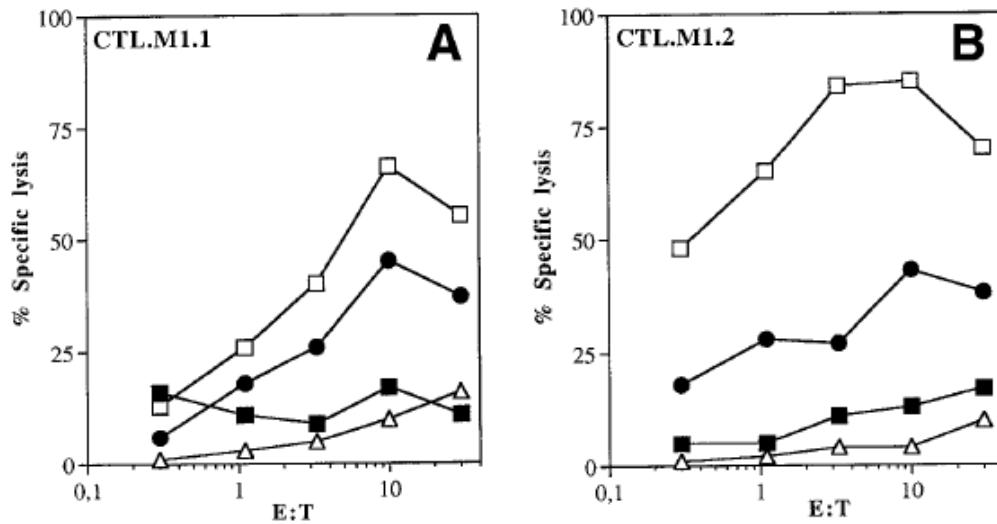


Figure 2. Lysis of cancer cells endogenously expressing MUC1 by CTL.M1.1 (A) and CTL.M1.2 (B). Human breast cancer cell line MCF-7 (HLA-A2 $^+$ /MUC1 $^+$), ovarian cancer cell line SK-OV-3 (HLA-A2 $^+$ /MUC1 $^+$), and the immortalized B-cell line Croft (HLA-A2 $^+$ /MUC1 $^+$) were used as targets in a standard ^{51}Cr -release assay. Croft cells were pulsed with the MUC1 peptides or an irrelevant HER-2/neu-derived peptide E75. (■) Croft + E75 peptide; (□) Croft + M1.1 (A) or M1.2 (B); (●) MCF-7; (Δ) SK-OV-3.

We have chosen to use the M1.1 peptide based on the large amount of data on the response to the MUC1 tandem repeat peptide, both in the human situation as well as in the mouse. Obviously only the human data are relevant for the clinical trials. We will use a HER-2/neu helper epitope (see below, not the PADRE helper epitope)

In the case of HER-2/neu, we have identified a novel CTL epitope HER-2 (9₄₃₅), which bound HLA-A2.1 with intermediate affinity (IC_{50} 74.6 nM). The peptide identified is: ILHNGAYSL. The .221(A2.1) cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human lymphoblastoid cell line .221, was used as target (peptide loaded) to measure activity of HLA-A2.1 restricted CTL [71]. The CTLs elicited following in vitro stimulation effectively killed HLA-A2.1⁺ tumor cells, showing that the antigen is appropriately processed by tumors (Fig. 3A). In addition, recognition of the tumor cell target was significantly inhibited by unlabeled (cold) target pulsed with HER-2 (9₄₃₅) peptide, but not by unlabeled targets either unpulsed or pulsed with a control HLA-A2.1 binding peptide (Fig. 3B).

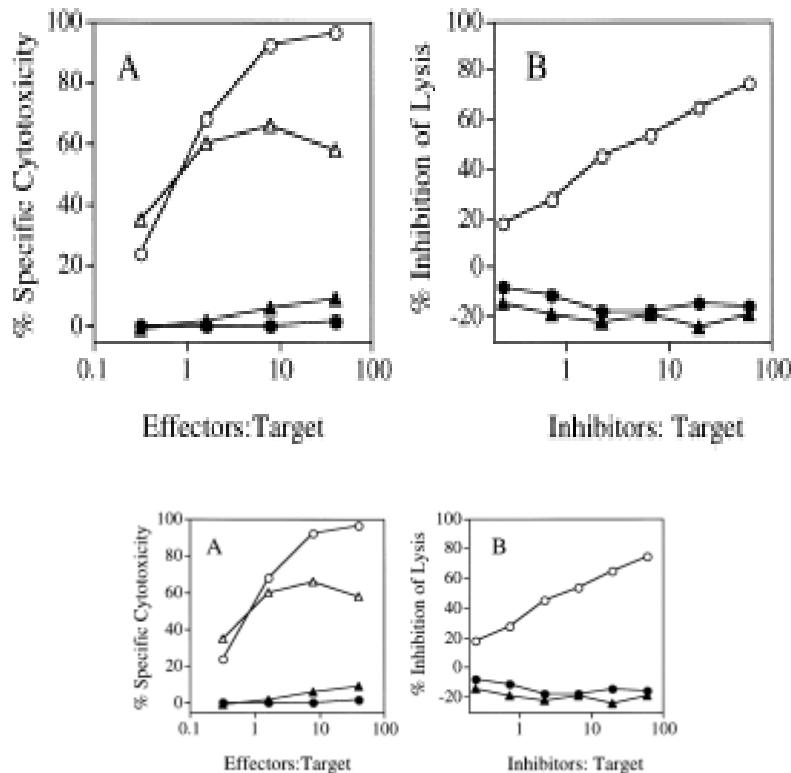


Figure 3. HER-2(9₄₃₅) specific CTL can kill tumor cells. (A) HER-2(9₄₃₅) specific CTL were used as effector cells to test for the lysis of the following target cell lines: o, .221A2.1 pulsed with HER-2(9₄₃₅); ●, .221A2.1 without peptide; Δ, SW403 (colon CA, A2⁺, HER-2/neu⁺); ▲, HT-29 (colon ca, A2⁺, HER-2/neu⁺). (B): Antigen specificity demonstrated by cold target inhibition assay. Lysis of ⁵¹Cr labeled SW403 cells at an effectors/target ratio of 10:1 by the HER-2(9₄₃₅) specific CTL was blocked at various Inhibitors/Target ratios by the following cold targets: o, .221A2.1 pulsed with HER-2(9₄₃₅); ▲, .221A2.1 pulsed with irrelevant A2.1 binding peptide (HBc₁₈₋₂₇); ●, .221A2.1 without peptide.

In addition to the class I epitopes described above, a promiscuous MHC class II epitope was defined for HER-2/neu using the algorithm tables published by Southwood et al. [58, 72]. The epitope identified is HER-2₈₈₃ (KVPIKWMALESILRRRF). It is important to show that these peptides represent true T cell epitopes that are relevant for the development of tumor immunotherapy. For these experiments autologous PBMCs or DCs were used as APCs and recombinant DNA derived intracellular domain or extracellular domain protein fragments of HER-2/neu were used as a source of antigen. The data in Fig. 4 show that four HER-2₈₈₃-reactive T cell lines proliferated well to HER-2/neu intracellular domain protein, which encompasses the HER-2₈₈₃ peptide but not to HER-2/neu extracellular domain (ECD), which lacks HER-2₈₈₃..

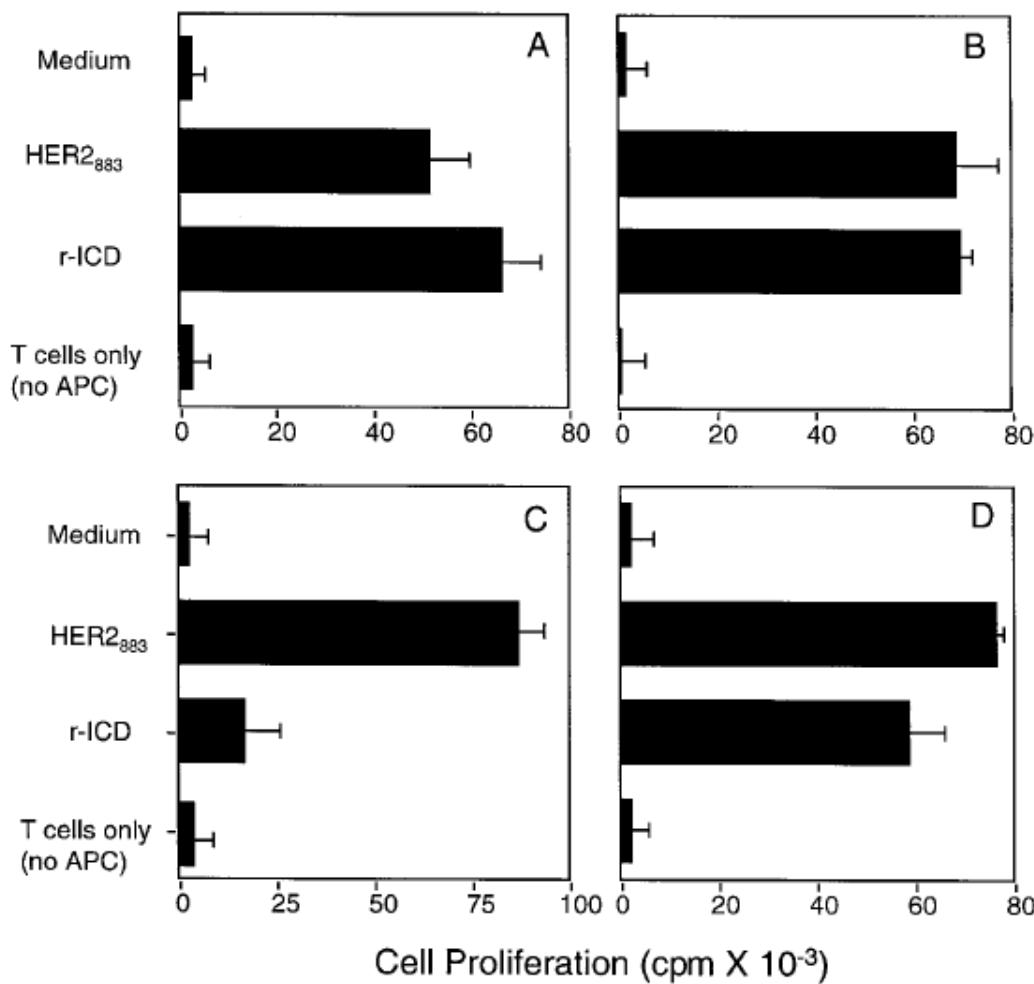


Figure 4. HER-2₈₈₃-specific CD4+ T cells can recognize recombinant HER-2/neu intracellular domain (r-ICD) protein presented by autologous DCs in the context of several HLA-DR alleles. The HER-2₈₈₃-reactive HTLs, TCL-7C (panel A, HLA-DR53 restricted), TCL-6D (panel B, HLA-DR4-restricted), a clone of TCL-1D (panel C, HLA-DR52-restricted), and TCL-1E (panel D, HLA-DR53 restricted), were tested for their capacity to proliferate to autologous DCs in the presence of HER-2₈₈₃ peptide (2.5 mg/ml) or recombinant HER-2/neu recombinant ICD protein (10 mg/ml). No significant proliferative response was observed against HER-2/neu ECD protein (data not shown). Values shown are the means of triplicate determinations; bars, SD.

1.9d Justification of vaccination strategy

1.9d.1 Peptide dose (1000ug): Over the last several years there has been extensive debate over the optimal dose of peptide in a variety of peptide immunization cancer clinical trials. Peptide doses have ranged from 50ug to 2500ug in various studies. Currently, the largest peptide vaccine clinical trial (E4697) utilizes a peptide dose of 1000ug. There are several published studies evaluating peptide vaccine dose-responses [66, 73], suggesting that 1000ug of peptide would be a reasonable vaccine dose for phase I/II clinical testing.

1.9d.2 GM-CSF suspended in Montanide ISA 51 as a vaccine adjuvant. The utility of GM-CSF suspended in montanide ISA 51 as an effective vaccine adjuvant has already been demonstrated in pre-clinical and clinical studies. Our own pre-clinical data (Fig 5) demonstrates a bell shaped dose-response curve for GM-CSF co-emulsified with 10ug

of *ova* peptide in montanide ISA 51. Two weeks after immunization, the optimal dose of GM-CSF in the mouse model appears to be 100ug. In humans, Slingluff et al. demonstrated successful peptide immunization using 225ug of GM-CSF suspended in montanide ISA-51[67]. Up to 80% of treated patients demonstrated effective immunization with melanoma differentiation antigen peptides. Our clinical data using 10, 50, 75 and 100 ug of GM-CSF suspended with peptides in Montanide ISA-51 failed to demonstrate effective generation of anti-peptide CTLs. In view of these data, we felt that it was reasonable to utilize the same dose of GM-CSF used by Slingluff [67] (225ug) with our current set of peptides. If successful, further studies will be performed attempting to generate a dose-response curve of GM-CSF and immunization efficacy similar to that of the mouse model.

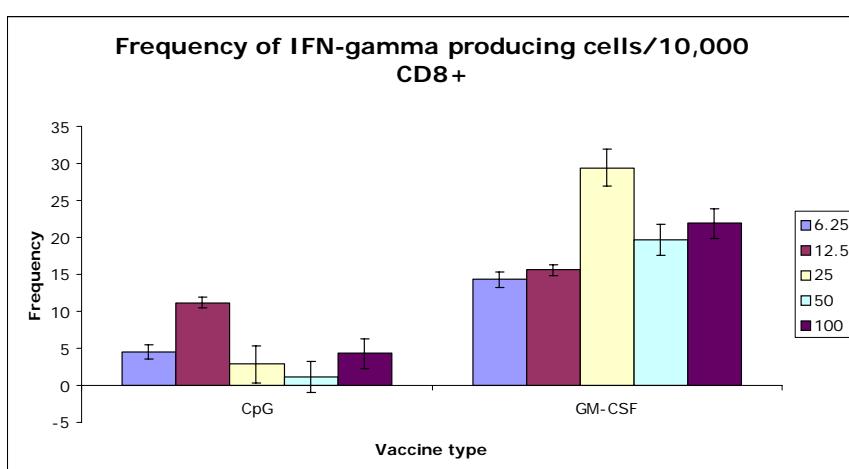


Fig. 5: C57BL6 mice (3 per group) were immunized with 10ug of *ova* peptide suspended in Montanide ISA51 and varying concentrations of CpG or GM-CSF .Represented are the frequencies of *ova* specific CTLs (IFN gamma ELISPOT) isolated from splenocytes on day 12 post immunization. Similar dose/response curves were observed in two other experiments.

1.9d.3 CpG suspended in Montanide ISA 51 as vaccine adjuvant. As described in section 15.7, the co-emulsification of peptide antigens with CpG and Montanide ISA-51 is an effective means of generation of peptide specific CTLs in a pre-clinical model. Our own data confirm these findings using non-transgenic mice immunized with *ova* peptide co-suspended with CpG in Montanide ISA 51 (Fig. 5). The dose of CpG used in the current study was empirically selected based on the results of a phase I clinical trial utilizing CpG (abbreviated as ISS in Fig. 6 legend) as an immune adjuvant for hepatitis B vaccine immunization in healthy volunteers. In this study, volunteers were immunized with an intramuscular injection of hepatitis B vaccine (20ug) mixed with CpG in one of the following amounts: 225ug, 650ug, 1000ug or 2250ug. A booster injection was administered 2 months later. Serologic data demonstrated (Fig 6) maximal immunization efficacy at CpG doses between 1000 and 2250ug. Based on these data suggesting a bell-shaped dose response curve for CpG (optimum may be between doses 1000ug and 2250ug) as well as our pre-clinical bell-shaped dose response curve, we elected to proceed with a CpG dose of 2000 ug.

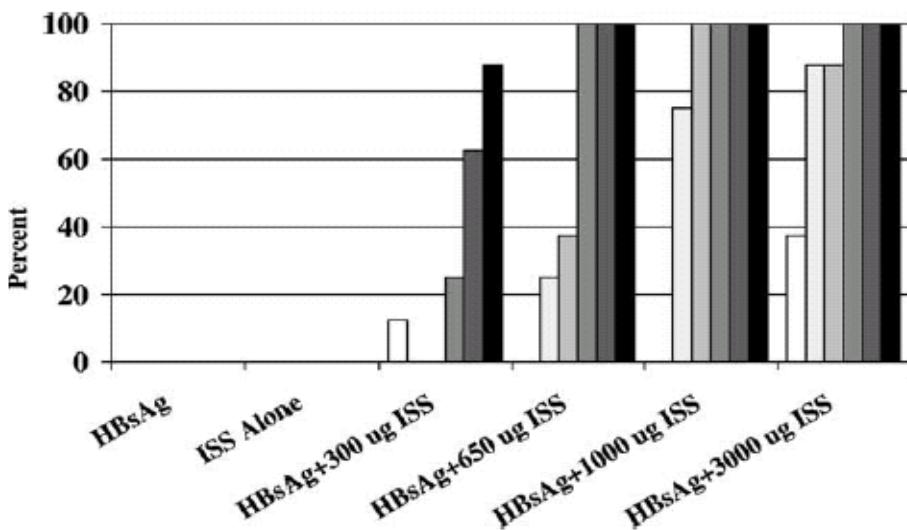


Figure 6: Proportion of participants achieving a protective antibody level ($\geq 10\text{mIU/mL}$) at various time points after immunization. Time points are (by increasing darkness of bar shade) 7 days after dose 1, 28 days after dose 1, 56 days after dose 1, 7 days after dose 2, 4 months after dose 2. CpG is designated as ISS.

The target population for this clinical trial, to whom the study findings will be generalized, are patients with a history of completely treated stage II or III breast adenocarcinoma that is MUC1 positive, currently off active therapy (with the exception of hormonal therapy) with no evidence of tumor relapse.

2.0 Goals

2.1 Primary Goal

To determine the safety and immunization efficacy of MUC1 and HER-2/neu peptide vaccines combined with CpG, GM-CSF or both, as immune adjuvants suspended in Montanide ISA-51.

2.2 Secondary Goal

To describe the impact of immunization on clinical outcomes in patients with MUC1 positive breast cancer. Clinical outcomes of interest will include: (1) **disease-free survival** defined as the time from registration to the documentation of a first failure where a failure is the recurrence (REC) of breast cancer or a diagnosis of a second primary cancer (NEWP); and (2) **overall survival** defined as the time from registration to death due to any cause.

3.0 Patient Eligibility

3.1 Pre-registration – Inclusion Criterion

3.1.1 Central pathology review submission. This review for MUC1 positivity is mandatory prior to registration to confirm eligibility (see Section 17.0). **It should be initiated as soon as possible after pre-registration.**

3.2 Registration - Inclusion criteria

3.2.1 Age ≥ 18 years.

3.2.2 Completed “standard first line therapy ONLY” (including adjuvant therapy) for breast cancer, clinical stage II and III (≥ 3 months prior to registration) and currently with no evidence of disease. NOTE: Current use of “anti-estrogen” therapy is allowed.

- 3.23 Histologically confirmed adenocarcinoma of the breast treated with surgery, adjuvant chemotherapy, and/or radiation therapy.
- 3.24 MUC1 positive breast cancer as determined by pre-registration central pathology review.
- 3.25 HLA-A2 positive.
- 3.26 The following laboratory values obtained \leq 14 days prior to registration:
 - Hemoglobin \geq 8.0 g/dL
 - Platelets \geq 75,000/ μ L
 - ANC \geq 1,500/ μ L
 - Creatinine \leq 2 x ULN
 - AST \leq 2 x ULN
- 3.27 Capable of understanding the investigational nature, potential risks and benefits of the study and capable of providing valid informed consent.
- 3.28 Willingness to return to Mayo Clinic Rochester, Scottsdale, or Jacksonville for treatment and study-related follow up. Study treatment will be administered only at the Mayo Clinic site where the patient was enrolled. Post-treatment study follow-up is allowed at the other participating Mayo Clinic sites.
- 3.29a Willingness to provide the blood specimens and complete the imaging studies as required by the protocol.

Note: The goals of this study include assessment of the biologic effects on surrogate markers of the agent(s) being tested and are, therefore, contingent upon availability of the blood specimens and completion of the required imaging studies.

- 3.29b Negative serum pregnancy test done \leq 7 days prior to registration, for women of childbearing potential only.

3.3 Registration - Exclusion criteria

- 3.31 ECOG performance status (PS) 3 or 4 (see Appendix I).
- 3.32 Uncontrolled infection.
- 3.33 Any of the following:
 - Known HIV infection
 - Other circumstances (i.e. concurrent use of systemic immunosuppressants and immunocompromising condition) that in the opinion of the physician renders the patient a poor candidate for this trial
- 3.34 Failure to fully recover from acute, reversible effects of prior breast cancer therapy regardless of interval since last treatment.
- 3.35 Any of the following:
 - Pregnant women
 - Nursing women unwilling to stop breast feeding

- Women of childbearing potential who are unwilling to employ adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], or abstinence, etc.)

NOTE: This study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown.

3.36 Other concurrent chemotherapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (utilized for a non-FDA-approved indication and in the context of a research investigation).

3.37 Radiographic evidence of disease at the time of enrollment.

3.38 Any prior invasive malignancies ≤ 5 years (with the exception of curatively-treated basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix).

3.39 Primary surgery for breast cancer **beyond 3 years** at time of registration.

4.0 Test Schedule

Tests and procedures	Active Monitoring Phase				
	Pre-Reg	≤ 14 days prior to reg	Prior to each subsequent treatment (q 4 weeks)	At 4 weeks after last treatment	Observation q 3 months for 2 years following registration
Central pathology review (see Section 17.1) ⁵	X				
History and assessment, wt, PS		X	X ^R	X	X
Height		X			
Hematology group: WBC, ANC, Hgb, PLT		X ^R	X ⁸	X ^R	
Chemistry group: total and direct bilirubin, AST, creatinine		X ^R	X ⁸	X ^R	
HLA class I and II typing ^R		At any time prior to reg			
Serum pregnancy test ¹		X			
Tumor typing ^R		At any time prior to reg			
Tumor evaluation by imaging study (x-ray, CT or PET)		X			X ²
DTH skin testing (common recall antigens) ^{3, R}		X	Prior to cycle 6 only		
Research blood specimens ⁷ See section 14.0		X	X ⁴		X ⁴
Acute toxicity evaluation ⁶			X	X	

1. For women of childbearing potential, must be obtained ≤ 7 days prior to registration.
2. Imaging will be performed per “standard of care” for patients and at the discretion of the treating physician
3. DTH skin testing will be performed using the same complement of antigens in routine use at the treatment site.

4. Research blood samples will be performed prior to registration, prior to cycles 3, 5 and 7 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration.
5. Tumor tissues will be stained for MUC1 and HER-2/neu mandatory central review after pre-registration but prior to registration.
6. Acute toxicity evaluations (physical exam and laboratory testing) will be performed for the purpose of evaluating potential immediate side effects of immunization.
7. Research blood specimens will be collected only if serum hemoglobin for the given collection is ≥ 10 g/dL. If hemoglobin is < 10 g/dL, research blood samples will be postponed until the next study office visit.
8. Research funded prior to cycles 2 and 5.

R. Research Funded

5.0 **Stratification Factors (*collected at registration*)**

Her-2/neu status: Positive vs. negative vs. unknown.

6.0 **Registration/Randomization Procedures**

6.1 Pre-Registration (Step 1)

6.11 To pre-register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The remote registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at (507)-284-4130 between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for remote pre-registration are available on the MCCC web page (<http://hsrwww.mayo.edu/ccs/training>) and detail the process for completing and confirming patient pre-registration. Users should refer to the section titled “Pre-Registration Components” for details on how to pre-register a patient to a study. At the time of pre-registration the patient will receive a MCCC patient identification number. This number is to be used when submitting tissue or blood samples, if applicable for the study (See Sections 14.0 and/or 17.0). Patient pre-registration via the remote system can be confirmed in any of the following ways:

- Contact the MCCC Registration Office (507)-284-4130. If the patient was pre-registered, the Registration Office staff can access the information from the centralized database and confirm the pre-registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

6.12 Prior to accepting the registration/randomization, the remote registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.2 Registration (Step 2)

6.21 To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The remote registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the

MCCC Registration Office at (507) 284-2753 between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for remote registration are available on the MCCC web page (<http://hsrwww.mayo.edu/ccs/training>) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the remote system can be confirmed in any of the following ways:

- Contact the MCCC Registration Office (507) 284-2753. If the patient was fully registered, the Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

- 6.22 A mandatory translational research component is part of this study. The patient will be automatically registered onto this component (Section 14.0).
- 6.23 A signed HHS 310 form must be on file in the Registration Office before an investigator may register any patients. Ongoing approval documentation must be submitted (no less than annually) to the Registration Office.
- 6.24 Prior to accepting the registration/randomization, the remote registration/randomization application will verify the following:
 - IRB approval at the registering institution
 - Patient eligibility
- 6.25 Treatment on this protocol must commence at Mayo Clinic Rochester, Scottsdale or Jacksonville under the supervision of a medical oncologist or hematologist.
- 6.26 Treatment cannot begin prior to registration and must begin ≤ 7 days after registration.
- 6.27 Pretreatment tests/procedures must be completed within the guidelines specified on the test schedule.
- 6.28 All required baseline symptoms must be documented and graded.
- 6.29 Study drug availability checked.

7.0 Protocol Treatment

7.1 For the purposes of this trial, patients will be recruited from the breast cancer practice of the Mayo Clinic Cancer Center. Patients who are undergoing regular follow-up visits by Mayo Clinic oncologists, are interested in this study, and fulfill all eligibility criteria will be offered enrollment. The patients' primary physicians, co-investigators in this study, will have the opportunity to offer the study to interested patients during their regularly scheduled follow-up visits. It is not expected that recruitment or advertisement materials will be used.

The patients who are enrolled will be assigned a 'study number' which will be used for their identification, and that of their data, throughout their participation in the clinical trial.

The Informed Consent process will take place during the patient's regular follow-up visits with their oncologists, co-investigators in the clinical trial. The informed consent interview will begin as part of the patient's regular follow-up visit. At that time, interested patients will be given information about the study, and if interested, will also receive a copy of the Informed Consent document. Patients will have the opportunity to discuss the details of the study during this visit or, more likely, will be given the consent form and offered to review the document at home and schedule a follow-up visit if they are interested in taking part on the study. This way the patients will have a chance to investigate and discuss the study on their own. If interested, the patients will set-up a 2nd visit with their oncologists specifically for the purpose of deciding on study participation. At that visit, all issues of concern for the patient will be addressed, eligibility reviewed and, if appropriate, the Consent Form will be signed.

7.2 As part of the registration process described in Section 6.0, the Mayo Clinic Cancer Center (MCCC) Remote Registration application will assign patients to arms A through C.

7.3 Treatment Schedules:

Arm A

	Agent	Dose	Route	RxDays	ReRx
Arm A	Montanide ISA-51	1.2mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1000ug			
	HER-2 peptide 1 (ILHNGAYSL)	1000ug			
	HER-2 peptide 2 (KVPIKWMALESILRRRF)	1000ug			
	GM-CSF	225ug			

Arm B

	Agent	Dose	Route	RxDays	ReRx
Arm B	Montanide ISA-51	1.2mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1000ug			
	HER-2 peptide-1 (ILHNGAYSL)	1000ug			
	HER-2 peptide-2 (KVPIKWMALESILRRRF)	1000ug			
	CpG	2mg			

Arm C

	Agent	Dose	Route	RxDays	ReRx
Arm C	Montanide ISA-51	1.2mL	subcutaneous injection in un-dissected LN region	Day 1 of Week 1	Q4 weeks (28-32 days) x 6 cycles
	MUC1 (STAPPVHNV)	1000ug			
	HER-2 peptide-1 (ILHNGAYSL)	1000ug			
	HER-2 peptide-2 (KVPIKWMALESILRRRF)	1000ug			
	GM-CSF	225ug			
	CpG	2mg			

7.4 Fifteen patients per arm (total of 45) will be randomly assigned to receive one of the three treatment schedules. Doses will not be escalated in any individual patient. It is not anticipated that there will be toxicity experienced with these regimens.

Vaccines will be prepared in a single vial and administered as multiple (2-3) subcutaneous injections in regions of un-disturbed axillary or inguinal lymph nodes. Each vaccine cycle will be administered into a single lymph node draining area. Subsequent vaccination cycles will be administered to other (rotating) undisturbed lymph node drainage sites.

The main risks are those of an allergic reaction to the components of the peptide vaccine (local or systemic). To minimize risk, patients will be observed by a registered nurse for 30 minutes following each immunization. On-site physicians will be available in the unlikely event that complications do occur. Risks due to phlebotomy will be minimized by ensuring that all patients will undergo phlebotomy by certified phlebotomists. All patients will be provided detailed contact information so that they are able to contact their treating physicians/co-investigators if they experience problems (medical or otherwise) while undergoing therapy in this study.

There are no antidotes available for the peptide vaccines used in this protocol. If patients develop symptoms as a result of the vaccines (e.g. allergic reactions), those patients will be treated accordingly.

The benefit to patients who undergo treatment in this study is unknown.

As IND sponsor, the Principal Investigator will monitor the protocol in accordance with 21 CFR 312, as indicated in portions of section 4.0, the test schedule; section 10.0, the adverse event reporting; and section 15, the drug information.

8.0 Dosage Modification Based on Adverse Events - Adjustments are based on adverse events observed since the prior dose.

ALERT: *ADR reporting may be required for some adverse events (See Section 10)*

→ → Use Common Terminology Criteria for Adverse Events (CTCAE) v3.0 unless otherwise specified ← ←			
CTCAE CATEGORY	ADVERSE EVENT	AGENT	DOSAGE CHANGE OR OTHER ACTION
AT TIME OF RETREATMENT			
ALLERGY/ IMMUNOLOGY	≥Grade 2 allergic reaction/ hypersensitivity	Montanide GM-CSF CpG Peptides	Discontinue vaccinations indefinitely and begin event monitoring.
	≥Grade 2 autoimmune reaction (excluding vitiligo)		Discontinue vaccinations indefinitely and begin event monitoring.
ALL OTHERS	≥Grade 3 Hematologic or ≥Grade 3 Nonhematologic (excluding alopecia) ≥Grade 2 neurologic		Discontinue vaccinations indefinitely and begin event monitoring.

9.0 Ancillary Treatment/Supportive Care

- 9.1 Patients should receive blood product support, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions.
- 9.2 Patients participating in this clinical trial are not to be considered for enrollment in any other study involving a pharmacologic agent (drugs, biologics, immunotherapy approaches, gene therapy) whether for symptom control or therapeutic intent.

10.0 Adverse Event (AE) Reporting and Monitoring

- 10.1 This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 for adverse event monitoring and reporting. The CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov/CTC3/ctc_ind_term.htm). All appropriate treatment areas should have access to a copy of the CTCAE v3.0.
- 10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE. Next, determine whether the event is expected or unexpected (refer to Section 15.0 and/or product literature) and if the adverse event is related to the medical treatment or procedure (see Section 10.12). With this information, determine whether an adverse event should be reported as an expedited report (see Section 10.2) or as part of the routinely reported clinical data.

Expedited adverse event reporting requires submission of a written report, but may also involve telephone notifications. Telephone and written reports are to be completed within the timeframes specified in Section 10.2. All expedited adverse event reports should also be submitted to the local Institutional Review Board (IRB).

10.12 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

- Definite - The adverse event is *clearly related* to the investigational agent(s).
- Probable - The adverse event is *likely related* to the investigational agent(s).
- Possible - The adverse event *may be related* to the investigational agent(s).
- Unlikely - The adverse event is *doubtfully related* to the investigational agent(s).
- Unrelated - The adverse event is *clearly NOT related* to the investigational agent(s)

10.2 Expedited Adverse Event Reporting Requirements

Phase I, II and III Studies (Investigational)

	Grade 4 or 5 ¹ Unexpected with Attribution of Possible, Probable, or Definite	Other Grade 4 or 5 or Any hospitalization during treatment ⁶	Secondary AML/MDS ²
Notify the Cancer Center IND Coordinator ³ within 24 hours	X		
Submit written report within 5 working days ⁴	X		
NCI/CTEP Secondary AML/MDS Report Form within 15 working days ⁵			X
Submit Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form within 5 working days. ⁶		X ⁶	

1. Includes all deaths within 30 days of the last dose of investigational agent regardless of attribution or any death attributed to the agent(s) (possible, probable, or definite) regardless of timeframe.
2. Reporting for this AE required during or after treatment.
3. Notify the Cancer Center IND Coordinator (Mayo Clinic - Rochester) by telephone (507) 284-0938 and/or submit a written event summary via fax to (507) 538-7164.
4. Use *Adverse Event Expedited Report – Single Agent or Multiple Agents* report form. Submit to the Cancer Center IND Coordinator (Mayo Clinic - Rochester) and to the Cancer Center Protocol Development Coordinator (PDC) for IRB reporting. The IND Coordinator will review the event in consultation with the IND holder and report to the Food and Drug Administration (FDA) as warranted by the event and required by U.S. federal regulations.
5. Submit per form-specified instructions and provide copy to Cancer Center IND Coordinator for review and FDA reporting (as warranted by the event) and the Cancer Center PDC for IRB reporting.
6. In addition to standard reporting mechanism for this type of event, submit information to the Cancer Center IND Coordinator and Cancer Center PDC. These persons will facilitate FDA and IRB reporting, respectively, as warranted by the event. If *Adverse Event Expedited Report – Single Agent or Multiple Agents* report form was completed, this form does not need to be completed.

10.3 Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per Common Terminology Criteria for Adverse Events (CTCAE) v3.0 grading unless otherwise stated in the table below:

CTCAE Category	Adverse event/Symptoms	Baseline	Each evaluation
Constitutional Symptoms	Fatigue (Asthenia, lethargy, malaise)	X	X
Dermatology/Skin	Injection site reaction		X
	Rash/desquamation	X	X
Pain	Musculoskeletal - Selects	X	X
	• Bone	X	X
	• Joint	X	X
	• Muscle	X	X

10.31 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.3:

10.311 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.312 Grade 3, 4, and 5 AEs and deaths within 30 days of the patient's last treatment, regardless of attribution to the study treatment or procedure, with the exception of signs or symptoms of definitely related to the patient's disease or disease progression.

10.313 Any death more than 30 days after the patient's last study treatment or procedure which is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.32 Refer to the instructions in the electronic data entry screens regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

**Information included at the request of the Department of Defense,
a financial sponsor of the study**

Reporting of serious or unexpected adverse events and unanticipated problems.

- Serious or unexpected adverse events and unanticipated problems can occur in any and all types of studies, not just experimental interventions or clinical trials.
- Include a definition of what constitutes an adverse event in the study.
 - For IND or IDE research include definitions as described in 21 CFR 312.32.
 - All IND protocols must address the following requirements.

“An adverse event temporally related to participation in the study should be documented whether or not considered to be related to the test article. This definition includes intercurrent illnesses and injuries and exacerbations of preexisting conditions. Include the following in all IND safety reports: Subject identification number and initials; associate investigator’s name and name of MTF; subject’s date of birth, gender, and ethnicity; test article and dates of administration; signs/symptoms and severity; date of onset; date of resolution or death; relationship to the study drug; action taken; concomitant medication(s) including dose, route, and duration of treatment, and date of last dose.”

- c. Describe agencies or offices to be notified with point of contact information in the event of a serious and unexpected adverse event.

All protocols should contain the following language regarding the HSRRB reporting requirements for adverse events and unanticipated problems. (Note that unanticipated problems can occur in a study that does not require a research/clinical intervention.)

“Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study and all volunteer deaths should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the Army Surgeon General’s Human Subjects Research Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012”

Refer to the “HSRRB Information Sheet for Investigators: Unanticipated Problems” for examples of unanticipated problems located on our website.

“The medical monitor for this project, Dr. Robert R. McWilliams, is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum the medical monitor should comment on the outcomes of the event or problem and in the case of a serious adverse event or death comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the HSRRB.”

The medical monitor will forward reports to the U.S. Army Medical Research and Material Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

11.0 Treatment Evaluation

- 11.1 For the purposes of this study, patients should be re-evaluated every 4 weeks during immunizations (treatment) and every 12 weeks during follow-up.
- 11.2 At the time of reevaluation, patients will be classified in the following manner:
 - 11.21 No evidence of disease (NED).

11.22 Breast cancer recurrence (PD). Local/regional breast cancer recurrence is defined as the development of tumor (except LCIS) in the ipsilateral breast (after lumpectomy); in the soft tissue/chest wall and/or skin of the ipsilateral chest wall; or tumor in the ipsilateral internal mammary, infraclavicular, or axillary nodes or soft tissue of ipsilateral axilla. Suspected tumor recurrence in the ipsilateral breast, chest wall structures or lower (level I ± II) axillary nodal areas must be confirmed by biopsy or cytology. Histologic or cytologic confirmation of tumor is recommended for internal mammary or infraclavicular/high axillary nodal recurrence. A distant recurrence is defined as development of tumor in areas other than the local/regional area that is documented by a positive cytology aspirate, biopsy, or imaging studies.

11.23 New primary (NEWP): A new primary is defined as the development of contralateral breast cancer or a second cancer other than squamous or basal cell carcinoma of the skin, carcinoma in situ of the cervix or LCIS of the breast that is histologically confirmed.

11.3 Further treatment after the documentation of a breast cancer recurrence or second primary cancer is left to the discretion of the treating physician.

12.0 Descriptive Factors: None.

13.0 Treatment/Follow-up Decision at Evaluation of Patient

13.1 Patients who have not recurred at time of their reassessment and have not experienced intolerable toxicity may continue protocol treatment at the same dose level for a maximum of 6 cycles or until progression of disease, a second primary or an intolerable adverse event occurs.

13.2 Patients who develop progression of disease, a second primary or intolerable toxicity will be removed from protocol treatment and go to the event monitoring phase of the study. Subsequent treatment is at the discretion of the treating physician.

13.3 Patients may refuse further protocol treatment at any time and go to the event-monitoring phase of the study.

13.4 If a patient is declared ineligible by the study team, on-study material, treatment evaluation forms, an End of Active Treatment/Cancel Notification Form must be submitted. No further follow-up after notification of ineligibility is required.

13.5 If a patient is declared a cancel by the study team before any study treatment is given, on-study material and End of Active Treatment/Cancel Notification Form must be submitted. No further follow-up is required.

13.6 If patient is found on central review to be MUC1 negative, the patient will be considered a cancel. The Pre-Registration Screening Failure Form must be submitted. No further data submission is necessary.

13.7 There will be no replacement of patients who discontinue or are removed from the protocol for any reason.

13.8 A patient is deemed a *cancel* if he/she is removed from study for any reason after pre-registration but prior to registration. The Pre-Registration Screening Failure Form must be submitted. No further data submission is necessary.

14.0 Correlative/Translational Studies

14.1 Description of Assays

Active vaccines for the immunotherapy of solid tumors have met with only limited success. It is our hypothesis that the causes of this failure are multifactorial and can be improved by the inclusion of stringent patient selection criteria, careful dose titration based on immunologic response monitoring, and correlation of immunologically based dosing parameters with clinical outcome. The following sections define the strategies that will be employed in this trial to evaluate immunologic response to MUC1, and HER-2 peptides.

14.11 Immune Responses to T Helper and CTL Epitopes

14.111 Elispot

Estimates of frequencies of peptide-specific, IFN γ - and IL-5-producing cytotoxic T lymphocytes and helper T lymphocytes will be obtained by ELISPOT assays following *in vitro* stimulation with peptide-sensitized stimulator cells [74, 75]. IL-5 production, rather than that of IL-4, will be assayed because of the increased signal:noise ratio [74]. CD8 $^{+}$ and CD4 $^{+}$ T cells will be positively selected by magnetic activated cell sorting (MACS, Miltenyi Biotech) from cryopreserved and thawed peripheral blood lymphocyte buffy coat. Antigen-presenting cells (APCs) will also be isolated from CD4 $^{-}$ /CD8 $^{-}$ cell population by MACS (beads and reagents purchased from Miltenyi Biotech). CD8 $^{+}$ and CD4 $^{+}$ responder T cells will be stimulated with irradiated APCs pulsed with the target peptides used for vaccination. After 5 days of co-culture, the responding cells will be diluted, titrated, and re-stimulated with APCs pulsed with target peptides for 24 hours in 96 well microtiter ELISPOT plates coated with IFN γ - or IL-5-specific capture antibody (ELISPOT Kit purchased from MABTECH, Stockholm, Sweden). The target peptides for re-stimulation include the peptide used for primary stimulation (MUC1 and HER-2 peptides) and a negative control peptide (YIGEVLVSV). The wells are washed and treated with ALP-conjugated secondary antibody and cytokine-producing spots detected using appropriate substrate (all reagents are provided in the kit). After stopping the reaction, the developed microtiter plates are shipped to Zellnet Consulting in New York for evaluation of number of spot-producing cells for each responder cell titration. All analyses are performed by the consulting firm and data provided electronically to the investigator. The difference between the frequency of spot-producing cells obtained with the target peptides and control peptide will determine the frequency of peptide-specific, cytokine-producing CD4 $^{+}$ or CD8 $^{+}$ T cells.

14.112 Tetramers

The estimation of frequencies of CTLs that recognize specific peptides bound to class I molecules became increasingly easier and more quantifiable with the construction and application of class I tetramers [11, 76, 77]. Class I MHC tetramers are composed of a complex of four HLA MHC class I molecules each bound to the specific peptide and conjugated with a fluorescent protein (MHC Tetramer-Streptavidin-Phycoerythrin (SA-PE)). We will use MUC1 M1.1 peptide (STAPPVHNV) and HER-2/neu peptide 9₄₃₅ (ILHNGAYSL). To detect epitope spreading, we will also use HER-2/neu peptide₃₆₉₋₃₇₇ (KIFGSLAFL). As a negative control, we will

use the multi-allele negative tetramer from Beckman Coulter (T01044). For positive control we will use the HLA-A0201 CMV PP65 tetramer (NLVPMVATV) from Beckman Coulter (T01009). Tetramers of HLA-A2 molecules are commercially available (Beckman Coulter). On the day of staining, test PBLs are thawed, washed, and resuspended in the manufacturer's recommended staining buffer (PBS) at 1×10^6 cells/ml. Tetramers and any additional antibodies (such as anti-CD8 or anti-CD3 conjugated to a different fluor such as FITC) are added to the cell volumes and incubated for 30 min at room temperature. The cell suspension is then washed with PBS and resuspended in PBS with 0.5% formaldehyde (Fixative Reagent) and analyzed by flow cytometry with FACSCAN instrumentation and CellQuest software (BD Biosciences); a minimum of 5×10^5 cells/sample are analyzed for accurate estimation of CD8 $^+$ CTLs with low frequencies. The analysis involves (1) gating on lymphocytes using forward and side-scatter; (2) gating on FITC-positive PBLs that stain with anti-CD3 or anti-CD8, and (3) analyzing the gated cells for PE and FITC staining. The frequency of doubly stained cells (tetramer $^+$ /CD8 $^+$) will be estimated for each of three replicate tubes for calculation of the mean frequency (\pm sd).

14.12 Antigenic Profiling

14.121 Expression of Class I HLA Antigens on tumor tissue.

Initial entry criteria require HLA-A typing of peripheral blood with subsequent confirmation of HLA class I antigen expression on tumor cells by immunohistochemistry. One of the mechanisms by which tumors are postulated to evade the immune response is by down regulation of classical HLA molecules necessary for antigen presentation.

14.122 Tumor Expression of MUC1

Tumor blocks will be used to determine the levels of expression of MUC1 on breast cancer tumor cells obtained at the time of most recent surgical resection. MUC1 expression will be determined by positive staining with one of several antibodies to MUC1 (HMFG-2, BC-2, or B27.29). Negative controls will be incubated with PBS instead of monoclonal antibody. Staining of cytoplasm and plasma membrane will be evaluated. Cells will be considered positive when at least one of these components is stained. Antibody staining patterns will be scored in a semi quantitative manner from +1 to +3.

14.13 Sample Schedule

14.131 Blood

100 mL of blood (about 7 tablespoons) will be collected (heparin) prior to registration, prior to cycles 3, 5 and 7 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration. Prior to each study blood collection a complete blood count will be performed. If the serum hemoglobin is less than 10.0, the study sample will not be collected. Study sample collection will be postponed for the next study visit.

14.132 Tumor

Tumor blocks will be collected from the patient's most recent surgery prior to study registration. Sections from the tumor blocks will be stained for MUC1. Any/all remaining tissue samples will be returned to the clinical file. Any/all excess samples will be destroyed.

14.14 Sample Preparation

14.141 Blood

Peripheral blood lymphocytes (PBLs) are enriched by flotation over Ficoll-Hypaque and frozen in aliquots in 10% DMSO for storage at -150°C. Percentages of CD4⁺ and CD8⁺ T cells, B cells, monocytes, and dendritic cells are estimated by flow cytometry with a panel of specific monoclonal antibodies. In addition, proliferation assays (3H-thymidine uptake) are performed to estimate T cell responses to polyclonal stimulus (phytohemagglutinin), target antigens (MUC1 and HER-2/neu) and a recall antigen (tetanus toxoid). These two sets of experiments are important for estimating the representation of individual lymphoid populations and evaluating overall T cell responsiveness. CD8⁺ (CTLs) and CD4⁺ (HTLs) are positively purified from cryopreserved and thawed PBLs by magnetic bead separation (Miltenyi Biotek). Additionally, serum will be collected and stored from each of these samples. Cells will then be frozen and stored at -150° for future use.

14.3 Delayed-type hypersensitivity (DTH) skin testing

Skin testing (baseline - prior to registration) will be coordinated through the Mayo Immunization/Allergy Clinic (L-15). A typical panel includes candida, mumps, PPD, and trichophyton. Other antigens may be substituted in the event of antigen unavailability. Patients will return for 1-2 follow-up measurements consistent with L-15 procedures. Patients must have a "positive" reaction to at least one of the antigens tested, to be considered eligible for participation. Patients with only "doubtful" or "negative" reactions will not be considered eligible.

15.0 Drug Information15.1 MUC-1 (STAPPVHNV) - *Investigational supply*

15.11 Other Names: epithelial membrane antigen (EMA), polymorphic epithelial antigen (PEM), DF3 antigen, Ca1, MAM-6, H23

15.12 Formulation and Storage: Samples will be vialed (glass vials with Teflon coated stoppers) as powder at a concentration of 1mg/vial and kept frozen at -20°C until use.

15.13 Drug Procurement and Accountability: to be purchased from Clinalfa

15.2 HER-2 Peptide-1 (ILHNGAYSL) - *Investigational supply*

15.21 Other Names: erbB2, neu

15.22 Formulation and Storage: Samples will be vialed (glass vials with teflon coated stoppers) as powder at a concentration of 1mg/vial and kept frozen at -20°C until use.

15.23 Drug Procurement and Accountability: to be purchased from Clinalfa

15.3 HER-2 Peptide-2 (KVPIKWMALESILRRRF) - *Investigational supply*

15.31 Other Names: erbB2, neu

15.32 Formulation and Storage: to be determined
Samples will be vialed (glass vials with teflon coated stoppers) as powder at a concentration of 1mg/vial and kept frozen at -20°C until use.

15.33 Drug Procurement and Accountability: to be purchased from Clinalfa

15.4 Montanide ISA-51 Adjuvant [MONTAN] - *Investigational supply*

15.41 Formulation and Storage

Montanide ISA-51 is an oil-based adjuvant product similar to Incomplete Freund's Adjuvant. When mixed with a water-based solution at a 1:1 w/w ratio, it forms a water-in-oil emulsion. It consists of highly purified oil, Drakol VR, and a surfactant, mannide oleate. Montanide ISA-51 is manufactured by Seppic, Inc., and is provided in amber glass ampoules or vials containing 3 mL of the solution. Montanide ISA-51 will be purchased from Seppic Inc.

15.42 Mode of Action

Acts to enhance immune response to vaccination; the precise mode of action is unknown.

15.43 Storage and Stability

The solution is stored at controlled room temperature. Exposure to cold temperatures may result in a clouded solution, which should be discarded. An expiration date is printed on the ampoule label.

15.44 Compatibilities/Incompatibilities

The oil may break down the rubber tip of the plunger on syringes; it is advisable to use a different syringe for each ampoule. Do not allow the Montanide ISA-51 to be in direct contact with the rubber tip of the plunger for more time than is necessary to withdraw the solution and inject it into the peptide vial. Fresh syringes will be needed to withdraw the emulsified vaccine from the vaccine vial. Once the emulsion is made, there is less interaction of the oil directly with the rubber tip of the plunger.

15.45 Drug Procurement and Accountability

Montanide ISA-51 will be purchased from Seppic Inc. The Cancer Center Pharmacy Shared Resource will store the drug and maintain records of inventory and disposition of all agent received.

15.5 GM-CSF (sargramostim, Leukine®)

15.51 Preparation and Storage

Liquid sargramostim (used in this study) is available in vials containing 500 mcg/mL (2.8×10^6 IU/mL) sargramostim. LEUKINE liquid should be refrigerated at 2-8°C (36-46°F). Do not freeze or shake. Do not use beyond the expiration date printed on the vial.

15.52 Known Potential Toxicities

Fever, chills, asthenia, malaise, numbness, increased sensitivity to touch, loss of balance, dizziness, rash, peripheral edema, dyspnea, headache, pericardial effusion, bone pain, arthralgia, nausea, vomiting, loss of appetite, developing or worsening of kidney or liver problems, difficulty breathing, shortness of breath, redness of the skin, facial flushing, rapid or irregular heartbeat or other heart problems, low blood pressure, myalgia, and serious allergic reactions such as a severe asthma attack.

15.53 Drug Procurement:

Leukine 500 mcg vials are available commercially. Drug will be purchased for this project using study grant funds. Patients will not be charged for the GM-CSF.

15.6 CpG-7909 (PF-3512676, Pfizer Pharmaceuticals, Inc)

15.61 Preparation and Storage: PF-3512676 (Injection) is formulated as a sterile phosphate buffered saline solution suitable for parenteral administration. This sterile and pyrogen-free solution contains no preservatives; vials are intended for single entry to prevent contamination. The drug product is packaged in clear, type I USP glass vials with Teflon-coated stopper closures and flip-caps. The drug product should be stored under refrigeration (2 to 8°C). Each vial contains 15mg/mL (1.2mL). Vials expire at 24 months.

15.62 Known potential toxicities: The list of reported serious adverse events with the use of CpG-7909 demonstrates the following toxicities:

15.621 **Related:** reactive follicular lymphatic hyperplasia.

15.622 **Possibly Related:** anemia, superior vena cava syndrome, dyspnea, malignant ascites, post-operative bleeding, hepatic failure, renal failure, post-operative wound infection, GI hemorrhage, prolonged coagulation time, bacteriemia, ureteric obstruction, congestive heart failure, DVT, vomiting, dehydration, vein compression, hydronephrosis, urinary retention, proctalgia, hypercalcemia, pleural effusion, subacute inflammatory demyelinating polyneuropathy, pelvic inflammatory disease, unstable angina, myocardial infarction, atrial fibrillation and grand mal seizures.

15.63 Drug Procurement: will be provided free of charge by Pfizer Pharmaceuticals, Inc..

15.7 Vaccine Preparation Instructions

15.71 General Vaccine Preparation Information

Emulsify the peptide(s)/GM-CSF or CpG mixture with Montanide ISA-51. Prepare the

vials as directed for each group below. Place the vial upside down in a tube platform holder of a vortex machine and vortex at highest speed for 12 minutes. This will minimize the amount of emulsion adhering to the inside surface of the vial. Because neither the peptide solution nor the Montanide ISA-51 contains preservatives or bacteriostatics, the prepared peptide vaccines should be administered as soon as possible.

15.711 Arm A

Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and thaw at room temperature. Remove a vial of liquid GM-CSF (500 mcg/mL) from the refrigerator and allow to reach room temperature. Withdraw 0.5 mL (250 mcg) of GM-CSF and add to one of the peptide vials. After the powder has dissolved, withdraw the mixture and add it to the 2nd peptide vial. Repeat the same procedure and add the solution to the 3rd peptide vial. Add 1.0 mL of Montanide ISA-51 to the 3rd peptide vial. Place the vial upside down in a tube platform holder of a vortex machine and vortex at the highest speed for 12 minutes. This will minimize the amount of emulsion adhering to the inside surface of the vial. Load two tuberculin syringes with equal volumes of this emulsion prior to use. Correct emulsification will be tested by carefully placing a small droplet of the emulsion on the surface of ice-cold distilled water (in a small 10 mL beaker) and observing that the droplet does not disperse after 2 minutes. Discard unused GM-CSF solution. Each syringe will be identified with the patient's name. The nurse will administer the vaccine mixture to the patient as soon as possible.

Arm B

Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and thaw at room temperature. Remove a vial of liquid CpG-7909 (15mg/mL) from the refrigerator and allow to reach room temperature. Withdraw 0.15mL of CpG-7909 (approximately 2.2 mg) and add to one of the peptide vials. Then add 0.35 mL sterile water to the vial. After the powder has dissolved, withdraw the mixture and add it to the 2nd peptide vial. Repeat the same procedure and add the solution to the 3rd peptide vial. Add 1.0 mL of Montanide ISA-51 to the 3rd peptide vial. Place the vial upside down in a tube platform holder of a vortex machine and vortex at the highest speed for 12 minutes. This will minimize the amount of emulsion adhering to the inside surface of the vial. Load two tuberculin syringes with equal volumes of this emulsion prior to use). Correct emulsification will be tested by carefully placing a small droplet of the emulsion on the surface of ice-cold distilled water (in a small 10 mL beaker) and observing that the droplet does not disperse after 2 minutes. Discard unused CpG solution. Each syringe will be identified with the patient's name. The nurse will administer the vaccine mixture to the patient as soon as possible.

Arm C

Remove one vial each of MUC1, HER-2 peptide 1, and HER-2 peptide 2 from the freezer and thaw at room temperature. Remove a vial of liquid GM-CSF (500 mcg/mL) from the refrigerator and allow it to reach room

temperature. Remove a vial of liquid CpG-7909 (15mg/mL) from the refrigerator and allow it to reach room temperature. Withdraw 0.15mL of CpG-7909 (approximately 2.2 mcg) and add to one of the peptide vials. Add 0.5mL (250 mcg) of GM-CSF to the mixture. After the powder has dissolved, withdraw the mixture and add it to the 2nd peptide vial. Repeat the same procedure and add the solution to the 3rd peptide vial. In the 3rd vial also add 1.2 mL of Montanide ISA-51 to the peptide vial. Place the vial upside down in a tube platform holder of a vortex machine and vortex at the highest speed for 12 minutes. This will minimize the amount of emulsion adhering to the inside surface of the vial. Load two to three tuberculin syringes with equal volumes of this emulsion prior to use). Correct emulsification will be tested by carefully placing a small droplet of the emulsion on the surface of ice-cold distilled water (in a small 10 mL beaker) and observing that the droplet does not disperse after 2 minutes. Discard unused GM-CSF and CpG-7909 solution. Each syringe will be identified with the patient's name. The nurse will administer the vaccine mixture to the patient as soon as possible.

15.8 Vaccine Administration Information

15.81 Dose Specifics

Each peptide vaccine will consist of a total volume of approximately 1.5 - 3 mL, containing the correct dose of the peptide(s), and/or GM-CSF and/or CpG. Be sure to confirm the proper cohort and dose level before preparing the product.

15.82 Administration

Vaccinations will be given subcutaneously on day 1 of each treatment cycle. Due to the large volume, each peptide vaccine is administered in 2 to 3 shots in a contiguous location the peptide vaccine should be injected in the vicinity of one of the major nodal basins. This basin must not have been dissected.

15.9 Vaccine Side Effects:

- 15.91 Because of the low dose of GM-CSF used and the slow release nature of the vaccine emulsion, side effects normally seen with systemic treatment doses of GM-CSF should not play a factor in this vaccination treatment. Expected side effects are related to the peptides and Montanide ISA-51. It is possible that the GM-CSF and CpG-7909 may potentiate the reaction seen at the injection site.
- 15.92 Dermatology/Skin: Injection site reaction, rare granuloma formation, possible development or worsening of pre-existing vitiligo, rash.
- 15.93 Hepatic: transient rises in liver transaminases.
- 15.94 Constitutional: Low-grade fever.

16.0 Statistical Considerations and Methodology

16.1 Study goals:

- **Primary goal:** to determine the safety and immunization efficacy of MUC1 and HER-2/neu peptide vaccines combined with CpG, GM-CSF or both, as immune adjuvants suspended in Montanide ISA-51.
- **Secondary goal:** to describe the impact of immunization on clinical outcomes in patients with MUC1 positive breast cancer.

16.2 The study design chosen for this proposal is a stratified randomized design. Toxicities will be carefully monitored and accrual will be suspended if 2 or more of the first six patients experience a grade 4 hematologic toxicity lasting for 5 or more days. In the event of at least two patients experiencing immunologic toxicity \geq grade 2 or any toxicity \geq grade 3 accrual will be temporarily suspended for the given treatment arm.

16.3 Accrual: Fifteen eligible patients with MUC1/HER-2 positive breast cancer and no evidence of disease will be randomized to each of the 3 treatment schedules. We anticipate 20% of the patients who pre-register to this study will be found not to have MUC-1 positive disease and 5% of the patients who do have MUC-1 positive disease will either cancel participation prior to starting treatment or will be found to be ineligible. As such, we anticipate preregistering 58 patient to obtain 45 eligible patients who will sign a consent form and start study treatment.

Patients will be assigned to treatment using a dynamic allocation procedure that balances the marginal distribution of type of dominant disease between treatments. The expected accrual rate for this study is approximately 15-20 patients per year at Mayo Clinic Rochester, 5-7 patients per year at Mayo Clinic Arizona and 5-7 patients per year at Mayo Clinic Florida. Enrollment is expected to extend approximately 2.5 years.

16.4 Study Endpoints:

16.41 Primary Endpoints

16.411 The immunologic parameters of interest are: (1) the percentage of CD4+ T cells, CD8+ T cells, B cells, monocytes, and dendritic cells in a patient's peripheral blood sample as estimated by flow cytometry with a panel of monoclonal antibodies and (2) the frequency of both peptide-specific IFN-gamma producing T cells and peptide-specific IL-5 producing T cells estimated by ELISPOT assays following *in vitro* stimulation with peptide-sensitized stimulator cells for the MUC1 and HER-2 peptides.

16.412 The number and severity of hematologic and non-hematologic toxicities reported using the NCI-CTC version 3.0 criteria

16.42 Secondary Endpoints

16.421 Disease-free survival is defined as the time from registration to the documentation of a first failure where a failure is the recurrence (REC) of breast cancer or a diagnosis of a second primary cancer (NEWP).

16.422 Overall survival is defined as the time from registration to death due to any cause.

16.43 Immunologic Parameters

- 16.431 All eligible patients who have completed one cycle of treatment are evaluable for the analysis of the immunologic parameters.
- 16.432 For each of the immunologic parameters, a plot of the parameter level against time will be constructed such that each patient is represented by a line connecting that patient's data points. These plots will enable visual assessment of patterns of change and variability within a parameter as well as a visual assessment of whether the immunologic parameters peak or fall at similar time points.
- 16.433 Also, for each of the immunologic parameters, a plot of the percent change from pre-treatment levels against time will be constructed such that each patient is represented by a line connecting that patient's data points. These plots will enable visual assessment of time trends within a parameter controlling for pretreatment levels.

16.44 Adverse Events

- 16.441 All eligible patients who received at least one vaccination are evaluable for toxicity.
- 16.442 The frequency of those hematologic and non-hematologic toxicities considered at least possibly related to treatment will be tabulated by severity.
- 16.443 The circumstances surrounding any treatment-related death will be reported.
- 16.444 As this is a pilot study, no formal hypothesis tests comparing treatment schedules are planned. An immunization strategy will be considered for further testing if at least 70% patients treated with that strategy had a ≥ 2 -fold increase in the percentage of vaccine-peptide specific CD8+ T cells during the course of treatment, with tolerable toxicity.
- 16.445 The principal investigator and study statistician will review the study every 3 months to identify potential accrual, toxicity, or endpoint problems. In addition, this study will be monitored by the Cancer Center Data Safety Monitoring Board. All patient related clinical data will be entered and maintained online, with reports generated as needed to comply with reporting guidelines.
- 16.446 It should be noted that representatives of the U.S. Army Medical Research and Merial Command are eligible to review research records as a part of their responsibility to protect human subjects in research.
- 16.447 If the protocol requires any modifications, deviations or termination prior to completion, all administrative activities will comply with the Protocol Review and Monitoring System of the Mayo Clinic Comprehensive Cancer Center. In addition, all local IRB communications, including deviations from protocol, will be forwarded to the Department of Defense HSRRB, upon local approval.

16.45 Inclusion of Minorities

This study will be available to all eligible patients, regardless of race or ethnic group. There is no information currently available regarding differential agent effects in subjects defined by gender, race, or ethnicity. The planned analyses will, as always, look for differences in treatment effect based on racial groupings. The sample sizes of this pilot study, however, are not sufficient to provide power for such subset analyses.

To predict the characteristics of patients likely to enroll in this trial we have reviewed registration to (non-North American Breast Cancer Intergroup) NCCTG breast cancer clinical trials by race. This revealed that roughly 3% of patients registered into cancer trials during the past five years could be classified as minorities, which would suggest that only 1 or 2 patients in the study sample are expected to be classified as minorities. This small sample precludes the possibility of a separate subset analysis beyond simple inspection of results for the 1 or 2 minority patients.

17.0 Pathology Considerations for Pre-registration Central Pathology Review

17.1 There will be a central review of MUC-1 expression.

17.11 The following materials are to be submitted.

- Central Testing of MUC1 Expression Form
- Surgical Pathology and Operative Report
- One H&E and 6 unstained slides

Slides should be placed in appropriate slide container and labeled with the protocol number, study patient number, and patient initials. All samples should be submitted to:

Mr. Michael Thompson
Mayo Clinic Rochester
221 4th Ave SW
Guggenheim 329
Rochester, MN 55905
Phone: (77)6-0963

Notify Michael Thompson by phone (507) 266-0963 or e-mail (thompson.michael4@mayo.edu) that samples have been shipped. Mike will log and ship samples to the Mayo Scottsdale lab for central review by Dr. Ann E. McCullough.

18.0 Records and Data Entry Procedures

18.1 Data Entry Timetable

Forms	Active-Monitoring Phase (Compliance with Test Schedule)				Event-Monitoring Phase ¹ (Completion of Active-Monitoring Phase)				At Each Occurrence			
	Pre-Reg	Initial material	Follow-up material		q.3 months until PD	At PD	After PD q.3 mos.	Death	ADR/AER	New Primary	Grade 4 or 5 Non-AER Reportable Events/ Hospitalization	Late Adverse Event
		≤2 weeks after registration	At each evaluation	At end of treatment								
On-Study Form		X										
Blood Specimen Submission Form ²		X	X									
Pathology Materials (see Section 17.0)	X											
Pre-reg Screening Failure Form ⁸	X											
Baseline Adverse Events Form		X										
Measurement Form		X	X	X								
Evaluation/Treatment Form			X ⁷	X								
Evaluation/Observation Form			X ⁵									
DTH Laboratory Form ³		X	X									
Interval Laboratory Form ⁴		X	X	X								
Adverse Event Form				X	X							
End of Active Treatment/ Cancel Notification Form			X ⁶		X							
Event Monitoring Form					X	X	X	X		X		X
Concurrent Treatment Form			X	X	X							
ADR/AER (See Section 10)									X			
Secondary AML/MDS Report Form (See Section 10)										X		
Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form (See Section 10.0)											X	

1. If a patient is still alive 2 years after registration, no further follow-up is required.
2. Research blood samples will be performed prior to registration, prior to cycles 3, 5, and 7 of therapy as well as every 3 months after conclusion of active therapy until 24 months following registration.
3. At baseline and prior to cycle 6 only.
4. At baseline, prior to each subsequent treatment and at 4 weeks after last treatment.
5. Complete at each evaluation during Observation (see Section 4.0).
6. Submit if withdrawal/refusal prior to beginning protocol therapy occurs.
7. Complete at each evaluation during Active Treatment (see Section 4.0).
8. Complete only if patient is NOT registered after he/she is pre-registered

19.0 Budget Considerations

- 19.1 Costs charged to patient: routine clinical care.
- 19.2 Tests and procedures to be research funded: HLA typing, tumor typing. DTH testing and serum pregnancy tests. Funding will be provided by the Department of Defense (DOD).

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Appendix I

ECOG PERFORMANCE STATUS

Grade

- 0 Fully active, able to carry on all pre-disease activities without restriction (Karnofsky 90-100).
- 1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work (Karnofsky 70-80).
- 2 Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50 percent of waking hours (Karnofsky 50-60).
- 3 Capable of only limited self-care, confined to bed or chair 50 percent or more of waking hours (Karnofsky 30-40).
- 4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20).
- 5 Dead

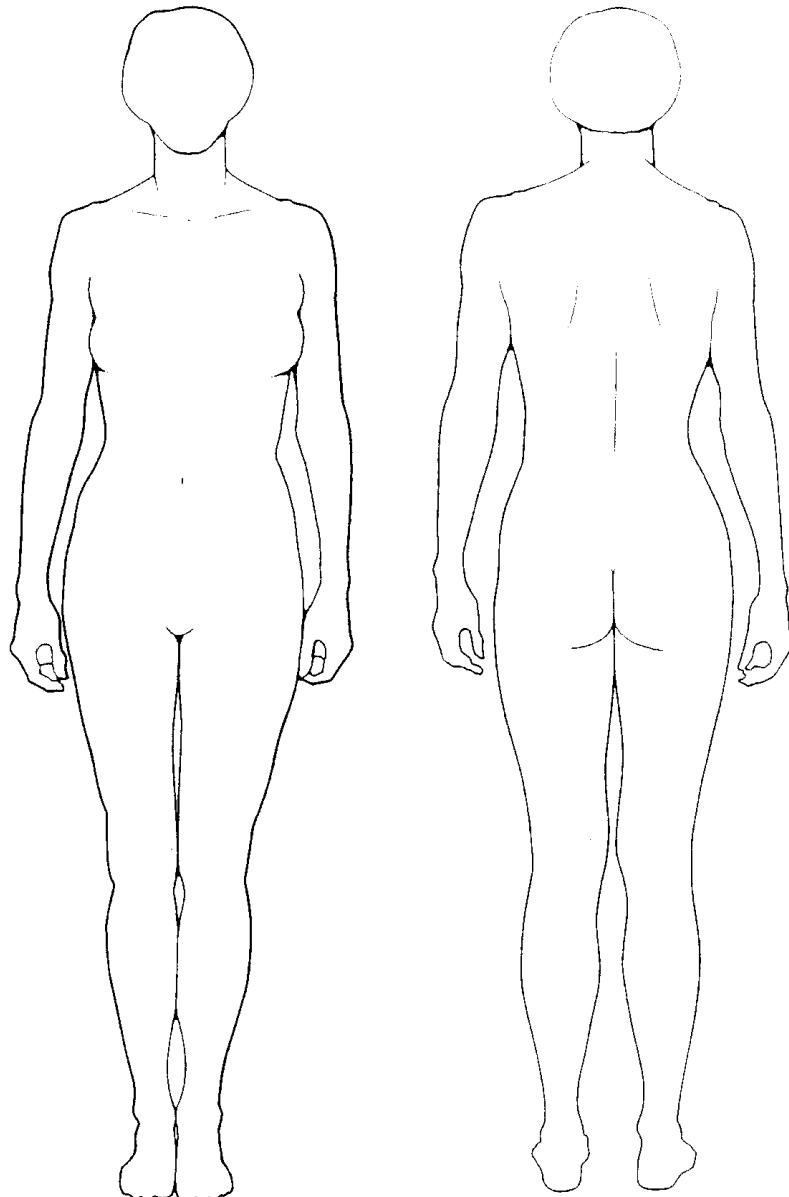
Appendix II

Site Injection Record

Protocol #: _____ Patient #: _____ Patient Initials: _____
F M L

Please indicate on the diagrams below all sites of vaccine injection (circle).

Date of determination: ____ / ____ / ____
M D Y



Anterior

Posterior

FORMS PACKET

MC0338; MUC1/HER-2/neu Peptide-Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

Contents:

- ✓ Pre-Registration Eligibility Checklist (8/28/08)
- ✓ Registration Eligibility Checklist (8/28/08)
- ✓ On-study form (6/18/08)
- ✓ Central testing of MUC1 expression form (8/25/08)
- ✓ Preregistration screening failure form (6/18/08)
- ✓ Baseline adverse events form (4/7/08)
- ✓ DTH laboratory form (6/18/08)
- ✓ Blood specimen submission form (7/23/08)
- ✓ Concurrent treatment form - Baseline (4/21/08)
- ✓ Concurrent treatment form – Active monitoring phase (4/21/08)
- ✓ Interval laboratory form (6/18/08)
- ✓ End of active treatment/cancel notification form (6/11/08)
- ✓ Evaluation/treatment form – Arm A (6/18/08)
- ✓ Evaluation/treatment form – Arm B (6/18/08)
- ✓ Evaluation/treatment form – Arm C (6/13/08)
- ✓ Evaluation/observation form (6/13/08)
- ✓ Adverse event form (4/7/08)
- ✓ Event monitoring form (4/7/08)
- ✓ Grade 4 or 5 non-AER reportable events/hospitalization form (4/7/08)

✓ designates revised/new forms

MAYO CLINIC CANCER CENTER
(MCCC)

Pre-Registration (Step 1) Eligibility Checklist

08/28/2008
Page 1 of 2

MC0338: MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application.

Has the patient ever been on a prior study entered through this Registration Office? Yes No

If yes: Prior study number _____; prior patient study ID number _____

Registration date (date on) (mm/dd/yyyy) ____/____/____

Patient study ID number (*provided at time of Pre-Registration*) _____

Member (participant sponsor) _____

Treating location _____

Treating physician _____

Institution patient number (local subject number) _____

IRB approval date (mm/dd/yyyy) ____/____/____

Person Completing Form:

Last Name: **(print)** _____ First Name: **(print)** _____

Phone: _____ Fax: _____ Email: _____

Patient initials (*last, first, middle*) _____
(*For Mayo Rochester patients, include first four letters of last name.*)

Gender (*check one*) Male Female Unknown

Date of birth (mm/dd/yyyy) ____/____/____

Zip code _____

Country of Residence _____

Race (*check all that apply*)

- White
- Black or African American
- Native Hawaiian or Other Pacific Islander
- Asian
- American Indian or Alaska Native
- Not reported: Patient refused or not available
- Unknown: Patient unsure

Method of payment (*check one*)

- PI (*Private Insurance*)
- MR (*Medicare*)
- MRP (*Medicare and Private Insurance*)
- MD (*Medicaid*)
- MM (*Medicaid and Medicare*)
- MVA (*Military or Veterans Sponsored, Not Otherwise Specified (NOS)*)
- MS (*Military Sponsored [including CHAMPUS & TRCARE]*)
- MV (*Veterans Sponsored*)
- SP (*Self pay [no insurance]*)
- NP (*No means of payment [no insurance]*)
- OTH (*Other*)
- UNK (*Unknown*)

Ethnicity (*check one*)

- Not Hispanic or Latino
- Hispanic or Latino
- Not reported: Refused or data not available
- Unknown: Unsure of their ethnicity

MCCC Pre-Registration (Step 1) Eligibility Checklist MC0338

08/28/2008

Patient study ID number (*provided at time of Pre-Registration*) _____

Page 2 of 2

Eligibility Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.**Inclusion Criteria**

Yes No NA

Central pathology review submission. This review for MUC1 positivity is mandatory prior to registration to confirm eligibility (see Section 17.0). It should be initiated as soon as possible after pre-registration.	_____
--	-------

All responses in above section must be “Yes.”Registration Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Yes No NA

Consent form signed and dated. Date of consent _____/_____/_____	_____
--	-------

Authorization for use and disclosure of protected health information signed and dated.	_____
--	-------

Date of authorization _____/_____/_____	_____
---	-------

All responses in above section must be “Yes” unless specified as “NA.”Pre-Registration

_____ Pre-Registration

Person registering _____ Registration Office specialist _____
Signature initialsPhysician _____
Signature M - D - Y

MAYO CLINIC CANCER CENTER
(MCCC)

Registration (Step 2) Eligibility Checklist

08/28/2008
Page 1 of 4

MC0338: MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application.

Has the patient ever been on a prior study entered through this Registration Office? Yes No

If yes: Prior study number _____; prior patient study ID number _____

Registration date (date on) (mm/dd/yyyy) ____/____/____

Patient study ID number (*provided at time of Pre-Registration*) _____

Member (participant sponsor) _____

Treating location _____

Treating physician _____

Institution patient number (local subject number) _____

IRB approval date (mm/dd/yyyy) ____/____/____

Person Completing Form:

Last Name: **(print)** _____ First Name: **(print)** _____

Phone: _____ Fax: _____ Email: _____

Patient initials (*last, first, middle*) _____
(*For Mayo Rochester patients, include first four letters of last name.*)

Gender (*check one*) Male Female Unknown

Date of birth (mm/dd/yyyy) ____/____/____

Zip code _____

Country of Residence _____

Race (*check all that apply*)

- White
- Black or African American
- Native Hawaiian or Other Pacific Islander
- Asian
- American Indian or Alaska Native
- Not reported: Patient refused or not available
- Unknown: Patient unsure

Method of payment (*check one*)

- PI (*Private Insurance*)
- MR (*Medicare*)
- MRP (*Medicare and Private Insurance*)
- MD (*Medicaid*)
- MM (*Medicaid and Medicare*)
- MVA (*Military or Veterans Sponsored, Not Otherwise Specified (NOS)*)
- MS (*Military Sponsored [including CHAMPUS & TRCARE]*)
- MV (*Veterans Sponsored*)
- SP (*Self pay [no insurance]*)
- NP (*No means of payment [no insurance]*)
- OTH (*Other*)
- UNK (*Unknown*)

Ethnicity (*check one*)

- Not Hispanic or Latino
- Hispanic or Latino
- Not reported: Refused or data not available
- Unknown: Unsure of their ethnicity

Eligibility Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Inclusion Criteria

	Yes	No	NA
Age \geq 18 years. Age = _____	_____	_____	_____
Completed “standard first line therapy ONLY” (including adjuvant therapy) for breast cancer, clinical stage II and III (\geq 3 months prior to registration) and currently with no evidence of disease. NOTE: Current use of “anti-estrogen” therapy is allowed.	_____	_____	_____
Histologically confirmed adenocarcinoma of the breast treated with surgery, adjuvant chemotherapy, and/or radiation therapy.	_____	_____	_____
MUC1 positive breast cancer as determined by pre-registration central pathology review.	_____	_____	_____
HLA-A2 positive.	_____	_____	_____
The following laboratory values obtained \leq 14 days prior to registration.	_____	_____	_____
Earliest laboratory test date ____/____/____; latest laboratory test date ____/____/____.	_____	_____	_____
NOTE: These dates pertain to the following labs only.	_____	_____	_____
• Hemoglobin \geq 8.0 g/dL Hemoglobin = _____	_____	_____	_____
• Platelets \geq 75,000/ μ L Platelets = _____	_____	_____	_____
• ANC \geq 1,500/ μ L ANC = _____	_____	_____	_____
• Creatinine \leq 2 x ULN Creatinine = _____; ULN = _____	_____	_____	_____
• AST \leq 2 x ULN AST = _____; ULN = _____	_____	_____	_____
Capable of understanding the investigational nature, potential risks and benefits of the study and capable of providing valid informed consent.	_____	_____	_____
Willingness to return to Mayo Clinic Rochester, Scottsdale, or Jacksonville for treatment and study-related follow up. Study treatment will be administered only at the Mayo Clinic site where the patient was enrolled. Post-treatment study follow-up is allowed at the other participating Mayo Clinic sites.	_____	_____	_____
Willingness to provide the blood specimens and complete the imaging studies as required by the protocol.	_____	_____	_____
<i>Note: The goals of this study include assessment of the biologic effects on surrogate markers of the agent(s) being tested and are, therefore, contingent upon availability of the blood specimens and completion of the required imaging studies.</i>	_____	_____	_____
Negative serum pregnancy test done \leq 7 days prior to registration, for women of childbearing potential only. If not a woman of childbearing potential or male (<i>check NA</i>) If a woman of childbearing potential - Negative serum pregnancy test date ____/____/____	_____	_____	_____

All responses in above section must be “Yes” unless specified as “NA.”

Exclusion Criteria

	Yes	No	NA
ECOG performance status (PS) 3 or 4 (see Appendix I). PS (0, 1, or 2) = _____	_____	_____	_____
Uncontrolled infection.	_____	_____	_____
Any of the following:	_____	_____	_____
• Known HIV infection	_____	_____	_____
• Other circumstances (i.e. concurrent use of systemic immunosuppressants and immunocompromising condition) that in the opinion of the physician renders the patient a poor candidate for this trial	_____	_____	_____
Failure to fully recover from acute, reversible effects of prior breast cancer therapy regardless of interval since last treatment.	_____	_____	_____

Exclusion Criteria (continued)

Yes No NA

Any of the following:	
<ul style="list-style-type: none"> • Pregnant women • Nursing women unwilling to stop breast feeding • Women of childbearing potential who are unwilling to employ adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], or abstinence, etc.) 	
<i>NOTE: This study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown.</i>	_____
Other concurrent chemotherapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (utilized for a non-FDA-approved indication and in the context of a research investigation).	_____
Radiographic evidence of disease at the time of enrollment.	_____
Any prior invasive malignancies \leq 5 years (with the exception of curatively-treated basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix).	_____
Primary surgery for breast cancer beyond 3 years at time of registration.	_____

All responses in above section must be "No."Registration Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Yes No NA

A mandatory translational research component is part of this study. The patient will be automatically registered onto this component (Section 14.0).	_____
Treatment on this protocol must commence at Mayo Clinic Rochester, Scottsdale or Jacksonville under the supervision of a medical oncologist or hematologist.	_____
Treatment cannot begin prior to registration and must begin \leq 7 days after registration.	_____
Pretreatment tests/procedures must be completed \leq 14 days prior to registration (see Section 4.0). Earliest pretreatment test/procedure date ____/____/____; latest pretreatment test/procedure date ____/____/____. NOTE: The earliest pretreatment test/procedure date must be less than or equal to the earliest laboratory test date and the latest pretreatment test/procedure date must be greater than or equal to the latest laboratory test date.	_____
Exceptions to the above dates:	
NOTE: HLA class I and II typing and Tumor typing; at any time prior to registration (see Section 4.0).	
All required baseline symptoms must be documented and graded.	_____
Study drug availability checked.	_____

All responses in above section must be "Yes."Stratification Factors (collected at registration)

Her-2/neu-status
 Positive
 Negative
 Unknown

Assigned Treatment

- A) peptide + Montanide ISA 51 + GM-CSF
- B) peptide + Montanide ISA 51 + CpG
- C) peptide + Montanide ISA 51 + CpG + GM-CSF

Person registering _____ Registration Office specialist _____
Signature initials

Physician _____
Signature M - D - Y

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

ON-STUDY FORM

page 2 of 3

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

Previous Radiotherapy: (check one) 1 Yes (describe below) 2 No

Site	Date (mm/dd/yyyy)	
	From	To

Previous Systemic Therapy for Breast Cancer: (check one) 1 Yes (describe below) 2 No

Therapy	Date (mm/dd/yyyy)		Was Therapy: (check one)	
	From	To	<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic
			<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic
			<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic
			<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic
			<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic
			<input type="checkbox"/> Adjuvant	<input type="checkbox"/> Metastatic

Any previous cancer? (check one) 1 Yes 2 No

If Yes: Site: _____

Date of Diagnosis: (mm/dd/yyyy) _____

Treatment: _____

Other Current Diseases: (check one) 1 Yes 2 No

If Yes, specify: _____

Estrogen/Progesterone Data

If bilateral disease this section applies to which breast: (check one) 1 Left 2 Right

Most recent result prior to registration:

Estrogen (check one) Date: (mm/dd/yyyy) _____

1 Positive 2 Negative 3 Unknown/no data

Progesterone (check one) Date: (mm/dd/yyyy) _____

1 Positive 2 Negative 3 Unknown/no data

HER2-Neu Method:

(check one) 3 Herceptest 9 Other, specify _____

Status: (check one) 0 0 4 Amplified
 1 1+ 5 Not amplified
 2 2+ 6 Strong positive
 3 3+

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



ON-STUDY FORM

page 3 of 3

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Complete in addition to above if Bilateral Tumor Laterality:

If bilateral disease this section applies to which breast: (check one) 1 Left 2 Right

Estrogen (check one) Date: (mm/dd/yyyy) ____/____/____-____
1 Positive 2 Negative 3 Unknown/no data

Progesterone (check one) Date: (mm/dd/yyyy) ____/____/____-____
1 Positive 2 Negative 3 Unknown/no data

HER2-Neu Method:

(check one) 3 Herceptest 9 Other, specify _____

Status: (check one) 0 0 4 Amplified
1 1+ 5 Not amplified
2 2+ 6 Strong positive
3 3+

Gender: (check one) 1 Male 2 Female

Actual Weight (kg): ____ . ____

Height (cm): ____ . ____

**CENTRAL TESTING OF MUC1 EXPRESSION FORM**

PLACE LABEL HERE

Protocol Number: MC0338Patient ID Number: _____ Patient Initials: L F M

Institution Number: _____ Clinic/Hospital: _____

Institution: _____ Date sent: _____

1. Date specimen obtained : (mm/dd/yyyy) ____/____/____-____

Slide ID: _____

Institution Contact Information: (Please Print)

Contact Person at Institution (CRA/Nurse): _____

Institution Name: _____

Street Address: _____

City: _____ State: _____ Zip Code: _____

Phone Number: _____

Fax Number: _____

E-mail Address: _____

2. MUC1 IHC+: (check one)

1 Yes2 No9 Not evaluable - another block needed

Criteria: any positivity in invasive carcinoma

3. % Positivity (in quartiles): (check one)

1 0-252 26-503 51-754 76-100

4. Pattern: (check all that apply)

 Diffuse Focal cytoplasmic Membranous

5. Intensity: (check all that apply)

 Dark Brown Tan; light brown

6. Histology: (check one)

1 Lobular2 Ductal3 Mixed4 Other, specify _____

7. Grade:

NBR grade: (check one) 1 2 3Tubules: (check one) 1 2 3Nuclear grade: (check one) 1 2 3Mitoses: (check one) 1 2 3

I. CRA/RN

II. Completed by the MCCC Pathology reviewer

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



**PREREGISTRATION
SCREENING FAILURE FORM**

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No

(if data are amended, please circle in red when using paper form)

Date aware of preregistration screening failure: (mm/dd/yyyy) ____/____/_____

Primary reason screening failed? (check one)

3 Did not meet eligibility criteria
1 Investigator decision
2 Patient decision
4 Other reason, specify _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

**BASELINE
ADVERSE EVENTS FORM**
ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Required Baseline Adverse Events from Section 10.0 of Protocol

CTC Adverse Events Term	MedDRA Code (v. 10.0)	CTC Adverse Event Grade
Fatigue (asthenia, lethargy, malaise)	10016256	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
Rash/desquamation	10037853	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
Pain - Selects		
- Bone	10006002	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
- Joint	10023222	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
- Muscle	10028411	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



DTH LABORATORY FORM

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Baseline: _____ OR Current Cycle Number: _____

Time point: (check one) 1 Baseline
2 Prior to cycle 6 or End of treatment

Mumps: _____ mm

Candida : _____ mm

Tetanus toxoid: _____ mm

Trichophyton: _____ mm

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



BLOOD SPECIMEN SUBMISSION FORM

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No

(if data are amended, please circle in red when using paper form)

Baseline or Current Cycle Number: _____

Time Point: (check one)

- 1 Baseline
- 2 Prior to Cycle 3
- 3 Prior to Cycle 5
- 4 Prior to Cycle 7
- 5 During observation

INSTRUCTIONS:

Complete this form for all patients and enter into the remote data entry system within 14 days of study entry/within 7 days of specimen collection. See Section 14 of the protocol for specimen requirements and shipment.

Was a research blood specimen collected? (check one)

1 Yes. If Yes, Date of collection: (mm/dd/yyyy) ____/____/____

2 No. If No, reason: _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



**CONCURRENT TREATMENT FORM
(BASELINE)**

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Evaluation Date: (mm/dd/yyyy) ____/____/____

Any of the following medications presently receiving or discontinued within 7 days of registration? (check one)

1 Yes 2 No (End Form)

Concomitant Treatment		
NSAIDS	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
ACE inhibitors	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Steroids	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Thyroid hormone	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Beta blocker	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



**CONCURRENT TREATMENT FORM
(ACTIVE MONITORING PHASE)**

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No

(if data are amended, please circle in red when using paper form)

Current Cycle Number: _____

Evaluation Date: (mm/dd/yyyy) ____/____/____

Has there been any change in medications since the previous report?

1 Yes 2 No (End Form)

Concomitant Treatment		
NSAIDS	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
ACE inhibitors	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Steroids	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Thyroid hormone	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No
Beta blocker	1 <input type="checkbox"/> Yes	2 <input type="checkbox"/> No

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



INTERVAL LABORATORY FORM

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No

(if data are amended, please circle in red when using paper form)

Baseline ___ OR Current Cycle Number: ___

		Test Result
Hematology	Date (mm/dd/yyyy)	____/____/_____
	TESTS	UNITS
	HGB	g/dL
	Platelets	K/uL or 10 ⁹ /L
	WBC	K/uL or 10 ⁹ /L
	ANC	K/uL or 10 ⁹ /L
	ALC	10 ³ /mm ³

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

END OF ACTIVE TREATMENT/CANCEL NOTIFICATION FORM
Submit Once Per Patient
ALL ITEMS MUST BE COMPLETED**Are data amended? (check one)** Yes No

(if data are amended, please circle in red when using paper form)

Last Date (any modality of) protocol therapy was given: (mm/dd/yyyy) ____/____/_____
 (date of last treatment dose on this study or date decision made not to initiate protocol treatment)

Off Treatment Date: (mm/dd/yyyy) ____/____/_____
 (date decision was made to end active treatment or not to initiate protocol treatment)

This patient will now go to: (check one)

(See Schema and Section 13.0
of the protocol)

1 Observation (follow test schedule and enter cycle data)
 2 Event Monitoring (follow Event Monitoring schedule)
 9 Off Study (cancels only)

Reason Treatment Ended (check one)	COMMENTS
1 <input type="checkbox"/> Treatment Completed Per Protocol Criteria	
2 <input type="checkbox"/> Patient Withdrawal/Refusal After Beginning Protocol Therapy	Specify:
24 <input type="checkbox"/> Patient Withdrawal/Refusal Prior To Beginning Protocol Therapy (cancel)	Specify:
3 <input type="checkbox"/> Adverse Event/Side Effects/ Complications	Specify:
4 <input type="checkbox"/> Disease Progression, Relapse During Active Treatment	Complete Event Monitoring Form
5 <input type="checkbox"/> Alternative Therapy	Specify:
6 <input type="checkbox"/> Patient Off-Treatment For Other Complicating Disease	Specify:
7 <input type="checkbox"/> Death On Study	Complete Event Monitoring Form
8 <input type="checkbox"/> Other	Specify:

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

**EVALUATION/TREATMENT FORM****ARM A****ALL ITEMS MUST BE COMPLETED**

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Use one form per cycle, one column per agent.

Current Cycle Number: _____

ECOG Performance Status: (check one) 0 1 2 3 4
(used for this cycle)

Agent	Montanide ISA-51 (MONTAN)	STAPPVHN (MUC1)	ILHNGAYSL (HER2-1)	KVPIKWMALESILRRRF (HER2-2)	GM-CSF (GMCSF)
Agent Start Date this cycle (mm/dd/yyyy)	____/____/_____	____/____/_____	____/____/_____	____/____/_____	____/____/_____
Total Dose this cycle (If agent was not given this cycle, enter 0 for total dose.)	mL	mcg	mcg	mcg	mcg

Was tumor assessed? (check one) 1 Yes 2 No

If Yes: Date of assessment: (mm/dd/yyyy) ____/____/_____

Objective status: (check one) 0 NED
 11 NEWP
 6 REC

Assessment method (check all that apply)

- CT
- Chest x-ray
- Mammogram
- Biopsy
- Cytology aspirate
- Other, specify _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

**EVALUATION/TREATMENT FORM****ARM B****ALL ITEMS MUST BE COMPLETED**

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Use one form per cycle, one column per agent.

Current Cycle Number: _____

ECOG Performance Status: (check one) 0 1 2 3 4
(used for this cycle)

Agent	Montanide ISA-51 (MONTAN)	STAPPVHNV (MUC1)	ILHNGAYSL (HER2-1)	KVPIKWMALESILRRRF (HER2-2)	CpG-7909 (CP7909)
Agent Start Date this cycle (mm/dd/yyyy)	____/____/_____	____/____/_____	____/____/_____	____/____/_____	____/____/_____
Total Dose this cycle (If agent was not given this cycle, enter 0 for total dose.)	mL	mcg	mcg	mcg	mg

Was tumor assessed? (check one) 1 Yes 2 No

If Yes: Date of assessment: (mm/dd/yyyy) ____/____/_____

Objective status: (check one)

0 <input type="checkbox"/>	NED
11 <input type="checkbox"/>	NEWP
6 <input type="checkbox"/>	REC

Assessment method (check all that apply)

<input type="checkbox"/>	CT
<input type="checkbox"/>	Chest x-ray
<input type="checkbox"/>	Mammogram
<input type="checkbox"/>	Biopsy
<input type="checkbox"/>	Cytology aspirate
<input type="checkbox"/>	Other, specify _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



EVALUATION/TREATMENT FORM

ARM C

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

Use one form per cycle, one column per agent.

Current Cycle Number: _____

ECOG Performance Status: (check one) 0 1 2 3 4
 (used for this cycle)

Agent	Montanide ISA-51 (MONTAN)	STAPPVHNV (MUC1)	ILHNGAYSL (HER2-1)	KVPIKWMALESILRRRF (HER2-2)
Agent Start Date this cycle (mm/dd/yyyy)	____/____/_____	____/____/_____	____/____/_____	____/____/_____
Total Dose this cycle (If agent was not given this cycle, enter 0 for total dose.)	mL	mcg	mcg	mcg

Agent	GM-CSF (GMCSF)	CpG-7909 (CP7909)
Agent Start Date this cycle (mm/dd/yyyy)	____/____/_____	____/____/_____
Total Dose this cycle (If agent was not given this cycle, enter 0 for total dose.)	mcg	mg

Was tumor assessed? (check one) 1 Yes 2 No

If Yes: Date of assessment: (mm/dd/yyyy) ____/____/_____

Objective status: (check one) 0 NED
 11 NEWP
 6 REC

Assessment method (check all that apply)

- CT
- Chest x-ray
- Mammogram
- Biopsy
- Cytology aspirate
- Other, specify _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____

ADVERSE EVENT FORM**ALL ITEMS MUST BE COMPLETED**

Pg. 1 of 2

Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

Current Cycle Number (*adverse events associated with this cycle*): _____

Evaluation Date: (mm/dd/yyyy) ____/____/____

CTC Adverse Event Term	MedDRA Code (v. 10.0) (must be completed)	CTC Adverse Event Grade (highest grade this cycle)	CTC AE Attribution Code (If Grade > 0)	Has an adverse event expedited report been submitted?* (Enter 1 for Yes or 2 for No)
		INCLUDE GRADE 0's	1 = Unrelated 2 = Unlikely 3 = Possible 4 = Probable 5 = Definite	

Required Adverse Events from Section 10.0 of Protocol

Fatigue (asthenia, lethargy, malaise)	10016256	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____
Injection site reaction	10022095	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____
Rash/desquamation	10037853	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 (death)	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____
Pain - Selects				
- Bone	10006002	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____
- Joint	10023222	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____
- Muscle	10028411	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5	_____

* See Section 10.0 of the protocol.

Protocol Number: MC0338

 Patient ID: _____ Patient Initials: _____
 L F M
 Institution Number: _____
 Institution: _____

ADVERSE EVENT FORM

ALL ITEMS MUST BE COMPLETED

Pg. 2 of 2

 Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

Current Cycle Number (adverse events associated with this cycle): _____

Were (other) adverse events assessed during this report period?

1 Yes, and reportable adverse events occurred
 2 No (Stop here)

3 Yes, but no reportable adverse events occurred (Stop here)


Adverse Events beyond those required in Section 10.0 of the protocol. Record grade 2 with attribution of possible, probable or definite and all grade 3, 4 and 5 regardless of attribution.**

Other CTC Adverse Event Terms not listed	MedDRA Code (v. 10.0) (must be completed)	CTC Adverse Event Grade (highest grade this cycle)	CTC AE Attribution Code (If Grade > 0)	Has an adverse event expedited report been submitted?* (Enter 1 for Yes or 2 for No)
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____
	□□□□□□□□□	2 3 4 5 (death)	1 2 3 4 5	____

* See Section 10.0 of the protocol.

** Both hematologic and nonhematologic Adverse Events must be graded on this form as applicable.

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____
 L F M
 Institution Number: _____
 Institution: _____

EVENT MONITORING FORM

ALL ITEMS MUST BE COMPLETED

Pg. 1 of 2

Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

Were you able to obtain any information about the patient since the last report?*

1 Yes. If Yes, complete rest of form.
 2 No. If No, date of last attempt to contact patient: (mm/dd/yyyy) ____/____/____-____ (End form)

Vital Status

1 Alive Date of last contact or death: (mm/dd/yyyy) ____/____/____-____

2 Dead

Primary Cause of Death: (check one) 1 Due to this disease 2 Due to other cause, specify _____
 4 Due to protocol treatment
 (adverse event related to treatment)

Disease Follow-up Status

Has the patient had a documented clinical assessment for this cancer (since submission of the last event monitoring form)?*

2 No. If No, Go to Notice of New Primary.
 1 Yes. If Yes, Cancer Follow-up Status Date: (mm/dd/yyyy) ____/____/____-____

Notice of First Relapse/Progression in the Event Monitoring Phase

Has the patient developed a first relapse or progression that has not been previously reported (in event monitoring phase)?

2 No 1 Yes. If Yes, Date of Relapse/Progression:** (mm/dd/yyyy) ____/____/____-____

Site(s) of Relapse/Progression: (check all that apply) Bone Brain
 Liver Skin
 Lung Chestwall
 Nodes Other, specify _____

Method (s) of Diagnosis: (check all that apply) Physical Exam Chest x-ray
 CT Patient correspondence
 MRI Other, specify _____
 ULT

Notice of New Primary

Has a new primary cancer or MDS (myelodysplastic syndrome) been diagnosed that has not been previously reported?

2 No 3 Unknown 1 Yes. If Yes, New Primary Cancer Date: (mm/dd/yyyy) ____/____/____-____
 Site of New Primary: _____

Late Adverse Event (post completion of active monitoring)

Has the patient experienced (prior to treatment for progression or relapse or a second primary, and prior to non-protocol treatment) any severe (grade ≥ 3) long term toxicity that has not been previously reported:

- Adverse events at least possibly attributed to treatment on this study.
- Death within 30 days of treatment.
- Death any time at least possibly treatment related.

2 No 3 Unknown/Not evaluated 1 Yes. If Yes, Submit page 2 of the Event Monitoring Form for Late Adverse Event Reporting.

*If this is the first event monitoring form check yes, enter cancer follow-up status date and complete the rest of the form.

**Submit documentation to verify PD.

PLACE LABEL HERE



Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____
 L F M
 Institution Number: _____
 Institution: _____

**EVENT MONITORING FORM
(LATE ADVERSE EVENT REPORTING)**
ALL ITEMS MUST BE COMPLETED

Pg. 2 of 2

Are data amended? (check one) Yes No
 (if data are amended, please circle in red when using paper form)

The CTC AE v.3.0 will be used to evaluate the following adverse events:

CTC Adverse Event Term	MedDRA Code (v. 10.0) (must be completed)	CTC Adverse Event Grade (Highest Grade)	CTC AE Attribution Code 1 = Unrelated 2 = Unlikely 3 = Possible 4 = Probable 5 = Definite	Late Adverse Event Onset Date (mm/dd/yyyy)
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____
	□□□□□□□□□□	3 4 5 (death)	1 2 3 4 5	____/____/_____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



NOTIFICATION FORM

Grade 4 or 5 Non-AER Reportable Events/Hospitalization

ALL ITEMS MUST BE COMPLETED

INSTRUCTIONS:

- Use this form to report all known information on non-AER reportable grade 4 or 5 adverse events or any hospitalization during active treatment.
- Verify reporting requirements listed within the study protocol, prior to entering into the remote data entry system.
- If AER has been submitted for this event do not enter this form.
- Fill out all information known.
- Enter into the remote data entry system within 5 working days of notification.
- These events must also be reported on the Nadir/Adverse Event Form.

Date membership CRA aware of event(s): (mm/dd/yyyy) ____/____/____

Name of Person Completing Form: _____ Phone: (____) ____-_____

Current Cycle Number: _____ Assigned Treatment Arm: _____

Event ≥ Grade 4: (check one) 1 Yes 2 No

Date of First Occurrence of Adverse Event (mm/dd/yyyy)	CTC Adverse Event Term <i>(only one event per line)</i>	CTC Adverse Event Grade	In your opinion, is this related to the study medication?*
____/____/____		<input type="checkbox"/> 4 <input type="checkbox"/> 5	4 <input type="checkbox"/> Yes: Unlikely 1 <input type="checkbox"/> Yes: Possible, probable, or definite 2 <input type="checkbox"/> No 3 <input type="checkbox"/> Unknown
____/____/____		<input type="checkbox"/> 4 <input type="checkbox"/> 5	4 <input type="checkbox"/> Yes: Unlikely 1 <input type="checkbox"/> Yes: Possible, probable, or definite 2 <input type="checkbox"/> No 3 <input type="checkbox"/> Unknown
____/____/____		<input type="checkbox"/> 4 <input type="checkbox"/> 5	4 <input type="checkbox"/> Yes: Unlikely 1 <input type="checkbox"/> Yes: Possible, probable, or definite 2 <input type="checkbox"/> No 3 <input type="checkbox"/> Unknown
____/____/____		<input type="checkbox"/> 4 <input type="checkbox"/> 5	4 <input type="checkbox"/> Yes: Unlikely 1 <input type="checkbox"/> Yes: Possible, probable, or definite 2 <input type="checkbox"/> No 3 <input type="checkbox"/> Unknown
____/____/____		<input type="checkbox"/> 4 <input type="checkbox"/> 5	4 <input type="checkbox"/> Yes: Unlikely 1 <input type="checkbox"/> Yes: Possible, probable, or definite 2 <input type="checkbox"/> No 3 <input type="checkbox"/> Unknown

*Answer YES if attribution is unlikely, possible, probable or definite; answer NO if unrelated; answer UNKNOWN if you are not sure.

Verify if expedited reporting (e.g. ADEERS) is required (see protocol), based on relationship to study treatment.

Hospitalization: (check one) 1 Yes 2 No

If Yes: Hospital Admission Date: (mm/dd/yyyy) ____/____/____

Reason(s) for Hospitalization:

1 Adverse Event, specify type and grade: _____2 Prophylactic, specify: _____3 Other reason, specify _____

PLACE LABEL HERE

Protocol Number: MC0338

Patient ID: _____ Patient Initials: _____

L F M

Institution Number: _____

Institution: _____



EVALUATION/OBSERVATION FORM

ALL ITEMS MUST BE COMPLETED

Are data amended? (check one) Yes No
(if data are amended, please circle in red when using paper form)

Use one form per cycle.

Current Cycle Number: _____

Weight (kg): _____. ____

(used for this cycle, round to the nearest tenth)

ECOG Performance Status: (check one) 0 1 2 3 4

(used for this cycle)

Observation*

Day 1 of this observation cycle: (mm/dd/yyyy) ____/____/____

Was tumor assessed? (check one) 1 Yes 2 No

If Yes: Date of assessment: (mm/dd/yyyy) ____/____/____

Objective status: (check one) 0 NED
11 NEWP
6 REC

Assessment method (check all that apply) CT
 Chest x-ray
 Mammogram
 Biopsy
 Cytology aspirate
 Other, specify _____

End of observation? (check one) 1 Yes 2 No

*When observation ends amend the last existing Evaluation/Observation Form by checking "Yes" for the End of observation question above.

MAYO CLINIC CANCER CENTER
(MCCC)

Pre-Registration (Step 1) Eligibility Checklist

08/28/2008
Page 1 of 2

MC0338: MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application.

Has the patient ever been on a prior study entered through this Registration Office? Yes No

If yes: Prior study number _____; prior patient study ID number _____

Registration date (date on) (mm/dd/yyyy) ____/____/____

Patient study ID number (*provided at time of Pre-Registration*) _____

Member (participant sponsor) _____

Treating location _____

Treating physician _____

Institution patient number (local subject number) _____

IRB approval date (mm/dd/yyyy) ____/____/____

Person Completing Form:

Last Name: **(print)** _____ First Name: **(print)** _____

Phone: _____ Fax: _____ Email: _____

Patient initials (last, first, middle) _____

(For Mayo Rochester patients, include first four letters of last name.)

Gender (*check one*) Male Female Unknown

Date of birth (mm/dd/yyyy) ____/____/____

Zip code _____

Country of Residence _____

Race (*check all that apply*)

- White
- Black or African American
- Native Hawaiian or Other Pacific Islander
- Asian
- American Indian or Alaska Native
- Not reported: Patient refused or not available
- Unknown: Patient unsure

Method of payment (*check one*)

- PI (*Private Insurance*)
- MR (*Medicare*)
- MRP (*Medicare and Private Insurance*)
- MD (*Medicaid*)
- MM (*Medicaid and Medicare*)
- MVA (*Military or Veterans Sponsored, Not Otherwise Specified (NOS)*)
- MS (*Military Sponsored [including CHAMPUS & TRCARE]*)
- MV (*Veterans Sponsored*)
- SP (*Self pay [no insurance]*)
- NP (*No means of payment [no insurance]*)
- OTH (*Other*)
- UNK (*Unknown*)

Ethnicity (*check one*)

- Not Hispanic or Latino
- Hispanic or Latino
- Not reported: Refused or data not available
- Unknown: Unsure of their ethnicity

MCCC Pre-Registration (Step 1) Eligibility Checklist MC0338

08/28/2008

Patient study ID number (*provided at time of Pre-Registration*) _____

Page 2 of 2

Eligibility Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.**Inclusion Criteria**

Yes No NA

Central pathology review submission. This review for MUC1 positivity is mandatory prior to registration to confirm eligibility (see Section 17.0). It should be initiated as soon as possible after pre-registration.	_____
--	-------

All responses in above section must be “Yes.”Registration Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Yes No NA

Consent form signed and dated. Date of consent _____/_____/_____	_____
--	-------

Authorization for use and disclosure of protected health information signed and dated. Date of authorization _____/_____/_____	_____
---	-------

All responses in above section must be “Yes” unless specified as “NA.”Pre-Registration

_____ Pre-Registration

Person registering _____ Registration Office specialist _____
Signature initialsPhysician _____
Signature M - D - Y

MAYO CLINIC CANCER CENTER
(MCCC)

Registration (Step 2) Eligibility Checklist

08/28/2008
Page 1 of 4

MC0338: MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application.

Has the patient ever been on a prior study entered through this Registration Office? Yes No

If yes: Prior study number _____; prior patient study ID number _____

Registration date (date on) (mm/dd/yyyy) ____/____/____

Patient study ID number (*provided at time of Pre-Registration*) _____

Member (participant sponsor) _____

Treating location _____

Treating physician _____

Institution patient number (local subject number) _____

IRB approval date (mm/dd/yyyy) ____/____/____

Person Completing Form:

Last Name: **(print)** _____ First Name: **(print)** _____

Phone: _____ Fax: _____ Email: _____

Patient initials (*last, first, middle*) _____
(*For Mayo Rochester patients, include first four letters of last name.*)

Gender (*check one*) Male Female Unknown

Date of birth (mm/dd/yyyy) ____/____/____

Zip code _____

Country of Residence _____

Race (*check all that apply*)

White
 Black or African American
 Native Hawaiian or Other Pacific Islander
 Asian
 American Indian or Alaska Native
 Not reported: Patient refused or not available
 Unknown: Patient unsure

Method of payment (*check one*)

PI (*Private Insurance*)
 MR (*Medicare*)
 MRP (*Medicare and Private Insurance*)
 MD (*Medicaid*)
 MM (*Medicaid and Medicare*)
 MVA (*Military or Veterans Sponsored, Not Otherwise Specified (NOS)*)
 MS (*Military Sponsored [including CHAMPUS & TRCARE]*)
 MV (*Veterans Sponsored*)
 SP (*Self pay [no insurance]*)
 NP (*No means of payment [no insurance]*)
 OTH (*Other*)
 UNK (*Unknown*)

Ethnicity (*check one*)

Not Hispanic or Latino
 Hispanic or Latino
 Not reported: Refused or data not available
 Unknown: Unsure of their ethnicity

Eligibility Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Inclusion Criteria

	Yes	No	NA
Age ≥18 years. Age = _____	_____	_____	_____
Completed “standard first line therapy ONLY” (including adjuvant therapy) for breast cancer, clinical stage II and III (≥3 months prior to registration) and currently with no evidence of disease. NOTE: Current use of “anti-estrogen” therapy is allowed.	_____	_____	_____
Histologically confirmed adenocarcinoma of the breast treated with surgery, adjuvant chemotherapy, and/or radiation therapy.	_____	_____	_____
MUC1 positive breast cancer as determined by pre-registration central pathology review.	_____	_____	_____
HLA-A2 positive.	_____	_____	_____
The following laboratory values obtained ≤14 days prior to registration.	_____	_____	_____
Earliest laboratory test date ____/____/____; latest laboratory test date ____/____/____.	_____	_____	_____
NOTE: These dates pertain to the following labs only.	_____	_____	_____
• Hemoglobin ≥8.0 g/dL Hemoglobin = _____	_____	_____	_____
• Platelets ≥75,000/µL Platelets = _____	_____	_____	_____
• ANC ≥1,500/µL ANC = _____	_____	_____	_____
• Creatinine ≤2 x ULN Creatinine = _____; ULN = _____	_____	_____	_____
• AST ≤2 x ULN AST = _____; ULN = _____	_____	_____	_____
Capable of understanding the investigational nature, potential risks and benefits of the study and capable of providing valid informed consent.	_____	_____	_____
Willingness to return to Mayo Clinic Rochester, Scottsdale, or Jacksonville for treatment and study-related follow up. Study treatment will be administered only at the Mayo Clinic site where the patient was enrolled. Post-treatment study follow-up is allowed at the other participating Mayo Clinic sites.	_____	_____	_____
Willingness to provide the blood specimens and complete the imaging studies as required by the protocol.	_____	_____	_____
<i>Note: The goals of this study include assessment of the biologic effects on surrogate markers of the agent(s) being tested and are, therefore, contingent upon availability of the blood specimens and completion of the required imaging studies.</i>	_____	_____	_____
Negative serum pregnancy test done ≤ 7 days prior to registration, for women of childbearing potential only. If not a woman of childbearing potential or male (<i>check NA</i>) If a woman of childbearing potential - Negative serum pregnancy test date ____/____/____	_____	_____	_____

All responses in above section must be “Yes” unless specified as “NA.”

Exclusion Criteria

	Yes	No	NA
ECOG performance status (PS) 3 or 4 (see Appendix I). PS (0, 1, or 2) = _____	_____	_____	_____
Uncontrolled infection.	_____	_____	_____
Any of the following:	_____	_____	_____
• Known HIV infection • Other circumstances (i.e. concurrent use of systemic immunosuppressants and immunocompromising condition) that in the opinion of the physician renders the patient a poor candidate for this trial	_____	_____	_____
Failure to fully recover from acute, reversible effects of prior breast cancer therapy regardless of interval since last treatment.	_____	_____	_____

Exclusion Criteria (continued)

Yes No NA

Any of the following:	
<ul style="list-style-type: none"> • Pregnant women • Nursing women unwilling to stop breast feeding • Women of childbearing potential who are unwilling to employ adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], or abstinence, etc.) 	
<i>NOTE: This study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown.</i>	_____
Other concurrent chemotherapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (utilized for a non-FDA-approved indication and in the context of a research investigation).	_____
Radiographic evidence of disease at the time of enrollment.	_____
Any prior invasive malignancies \leq 5 years (with the exception of curatively-treated basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix).	_____
Primary surgery for breast cancer beyond 3 years at time of registration.	_____

All responses in above section must be "No."Registration Check - Answer questions below (yes/no). All requirements must be confirmed. All dates are to be *mm/dd/yyyy*.

Yes No NA

A mandatory translational research component is part of this study. The patient will be automatically registered onto this component (Section 14.0).	_____
Treatment on this protocol must commence at Mayo Clinic Rochester, Scottsdale or Jacksonville under the supervision of a medical oncologist or hematologist.	_____
Treatment cannot begin prior to registration and must begin \leq 7 days after registration.	_____
Pretreatment tests/procedures must be completed \leq 14 days prior to registration (see Section 4.0). Earliest pretreatment test/procedure date ____/____/____; latest pretreatment test/procedure date ____/____/____. NOTE: The earliest pretreatment test/procedure date must be less than or equal to the earliest laboratory test date and the latest pretreatment test/procedure date must be greater than or equal to the latest laboratory test date.	_____
Exceptions to the above dates:	
NOTE: HLA class I and II typing and Tumor typing; at any time prior to registration (see Section 4.0).	
All required baseline symptoms must be documented and graded.	_____
Study drug availability checked.	_____

All responses in above section must be "Yes."Stratification Factors (collected at registration)

Her-2/neu-status
 Positive
 Negative
 Unknown

Assigned Treatment

- A) peptide + Montanide ISA 51 + GM-CSF
- B) peptide + Montanide ISA 51 + CpG
- C) peptide + Montanide ISA 51 + CpG + GM-CSF

Person registering _____ Registration Office specialist _____
Signature _____ initials _____

Physician _____
Signature _____ M - D - Y

Memo

Date: August 29, 2008

To: MC0338 Study Team

From: Jane Milburn
Protocol Development Coordinator

Re: **Revised Activation Memo -**

**MC0338; MUC1/HER-2/neu Peptide-Based Immunotherapeutic Vaccines for
Breast Adenocarcinomas**

Study Chair: Dr. Markovic

The above study is now activated for patient enrollment.

A protocol and forms packet are enclosed. Please note the following information regarding the pre-registration component.

- Submit 1 H&E and 6 unstained slides to Dr. Sandra Gendler in Mayo, AZ (c/o Cathy Madsen at the following address) by FedEx and a tracking number sent to Sandra Gendler and Cathy Madsen.

Cathy S. Madsen
Senior Research Technologist for Dr. Sandra Gendler
Mayo Clinic Scottsdale
Johnson Research Building SCJ 2-221
13400 E. Shea Blvd.
Scottsdale AZ 85259
- The slides from patients enrolled at MCR will be sent by Christie Maszk (Pathology Coordinator). The Central Testing of MUC1 Expression Form will be filled out (top portion) and scanned and sent by e-mail to Dr. Ann McCullough (McCullough.ann@mayo.edu), Sandra Gendler (Gendler.sandra@mayo.edu), Cathy Madsen (cathy.madsen@mayo.edu) and Jennifer Roedig (roedig.jennifer@mayo.edu).
- The slides from patients enrolled at MCS will be sent by Leslie Dixon to Cathy Madsen. The Central Testing of MUC1 Expression Form will be filled out (top portion) by Jennifer Roedig and scanned and sent by e-mail to Dr. Ann McCullough (McCullough.ann@mayo.edu), Sandra Gendler (Gendler.sandra@mayo.edu), Cathy Madsen (cathy.madsen@mayo.edu) and Jennifer Roedig (roedig.jennifer@mayo.edu).
- The slides will be stained in Dr. Gendler's lab.
- Cathy will log and forward the slides to Dr. Ann McCullough in Scottsdale for central pre registration review of MUC1 expression.
- The completed Central Testing of MUC1 Expression Form will be faxed to the Registration Office at 4-0885 and to the Pathology Coordinator, Christine Maszk, at 4-9628 by Eleanor Gray of Dr. McCullough's lab as soon as possible after test results become available.
- Slides and correlating paperwork will be returned to Cathy Madsen for Dr. Gendler's lab.

If you have any questions, please feel free to contact me.

Consent Form for Participation in a Research Study

TITLE: MC0338, "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas"

IRB #: 782-05 00

RESEARCHER: Dr. S. N. Markovic and colleagues

PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD: October 25, 2007

THIS FORM APPROVED: October 25, 2007

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

We are inviting you to participate in this study because of your history of resected breast cancer for which you have already received standard therapy and have no evidence of relapse. Your good overall health and no other history of cancer make it possible for us to offer you participation on this clinical study.

This study is being done to evaluate three different preparations of a breast cancer vaccine to stimulate anti-cancer (T-cell) immune responses and any side effects associated with these vaccinations. The breast cancer vaccine (MUC-1/HER-2/neu peptides) will be combined with one of two immune boosting agents (CpG or GM-CSF) or with immune boosting agents together. These immune boosting agents are believed to be able to make the vaccine more effective. Whether or not this will protect you from breast cancer is still unknown.

The breast cancer vaccine and one of the immune boosting agents (CpG) have not been approved by the Food and Drug Administration (FDA) for commercial use; however, FDA has permitted their use in this research study. GM-CSF is commercially available for use in clinical practice. Laboratory experiments have shown that both of these immune boosting agents are able to make the vaccines more effective in generating an immune response. In laboratory animals, both agents are very effective in boosting anti-

tumor immune responses. However, in clinical trials, neither GM-CSF or CpG have shown effects against cancer, but they both seem to improve the effectiveness of some vaccines. One of the goals of this study is to determine if the unique application of GM-CSF and/or CpG in this study, based on our laboratory data, will make them more likely to improve vaccine immunity.

This study is sponsored by the Department of Defense.

How many people will take part in this research study?

The plan is to have up to 45 people take part in this study at Mayo Clinic Rochester, Jacksonville, and Arizona. Up to 60 people may be screened to find enough eligible people to begin the study.

What will happen in this research study?

Before you enter the study, you will have a physical examination and blood tests to make sure that you qualify to take part in this study. About 6 tablespoons of blood will be taken for testing.

If you qualify to take part, you will have blood taken for immunologic testing (to see how your immune system is working) for the study as well as a skin test (allergy test). You will then be randomly assigned (as in the toss of the dice) to be treated with one of three breast cancer vaccines combinations.

Each vaccination will consist of one or two subcutaneous (under the skin) injections of about one half teaspoonful (2ml) of the cancer vaccine and boosting agent combination. The vaccine will be given with Montanide ISA 51, which is an oil that is mixed with the vaccine so that the vaccine can be released into the body slowly. The vaccine will be injected under the skin in areas where there has been no surgery. Usual areas of vaccination include the skin of the upper arms and legs. Vaccinations will be repeated every 4 weeks for 6 months. Before every vaccination, you will be seen by your doctor, examined and 6 tablespoons of blood will be collected to study the development of the anti-cancer immune response. Skin tests will be done prior to vaccinations #6 (see the following table). Evaluations for the cancer (body scans) will be done if and when your doctor thinks it is necessary.

Pre-Study	<ul style="list-style-type: none"> • Routine¹ and research blood tests • Cancer evaluation (scans) • Physical examination • Skin test (Mayo Clinic Rochester, only)
Vaccination #1 (month 1)	<ul style="list-style-type: none"> • Vaccine treatment
Vaccination #2 (month 2)	<ul style="list-style-type: none"> • Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #3 (month 3)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment
Vaccination #4 (month 4)	<ul style="list-style-type: none"> • Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #5 (month 5)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Vaccine treatment
Vaccination #6 (month 6)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment • Skin test (Mayo Clinic Rochester, only)
Every 3 months after the first six months until 5 years after first vaccination	<ul style="list-style-type: none"> • Physical examination • Routine blood tests • Cancer evaluation (scans)
Every 3 months after the first six months until 2 years after first vaccination	<ul style="list-style-type: none"> • Research bloods

1. Routine blood tests include: complete blood count and blood chemistries.

How long will I be in this research study?

You will be on the study for 6 months, and you will be seen in follow-up until 5 years after your first vaccination.

Are there reasons I might leave this research study early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the investigators or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

No. Your samples will be used as described for this study, and then will be destroyed.

What are the risks of this research study?

You will be closely watched by the study team for any side effects. If side effects happen, the study team will take the necessary steps to treat them. This may include stopping the medication and/or stopping the study.

The possible side effects of the following: MUC-1/HER-2/neu, breast cancer vaccine, Montanide ISA 51 (the oil), CpG (immune booster), and GM-CSF (immune booster) include:

Common:

- Injection site reactions: discomfort, rash, redness, firmness, warmth, bleeding, tenderness to touch, numbness, tingling, itching.
- Systemic reactions: skin rash, itching, sweating, muscle aches, joint aches, fatigue.

Rare:

- Injection site reactions: pain, ulceration.
- Systemic reactions: low blood counts, difficulty breathing, heart problems, blood clots, seizures, infection, kidney and liver problems, headache, stomach pain, cough.

Although many of these reactions are similar to vaccines that you may have received in the past, the listed side effects that could happen might be more severe. However, we expect that all of these reactions will be very mild. Treatment for these reactions will depend on the type and severity of the reaction. Treatments will be directed at suppressing the immune reaction to the vaccine and may range from mild anti-allergic/anti-inflammatory treatments (for example topical hydrocortisone or Motrin) to more powerful anti-inflammatory therapy including corticosteroids. If severe reactions happen, they may require hospitalization and possibly even minor surgery (severe local skin reactions).

When GM-CSF has been given at higher doses as a daily injection the following side effects have also been reported: diarrhea, general weakness, fever, chills, nausea, vomiting, loss of appetite, headache, pain in the bones, joints and muscles. Most of the symptoms were mild or moderate in severity and were less after taking acetaminophen (Tylenol). Other side effects which happened very rarely were: difficulty breathing, rapid or irregular heart beat or other heart problems, swelling. Even less common, reported side effects have been the following: 1) increased white cells in the lungs with breathing

problems; 2) a syndrome of shortness of breath, low oxygen in the blood, redness in the skin, low blood pressure and dizziness when you stand up or a loss of balance and partial loss of consciousness; 3) serious allergic reactions (like a very severe asthma attack); 4) blood clotting; 5) facial flushing; 6) kidney or liver problems; 7) worsening of fluid accumulation in the arms, legs, lungs or around the heart which may cause problems with breathing or heart failure; 8) patients with heart, lung, kidney or liver problems may have worsening of their symptoms following GM-CSF; and 9) nerve toxicity (weakness, shooting pains, numbness, increased sensitivity to touch, loss of balance, dizziness)

Skin testing: The risks and discomfort of skin testing are minimal and usually limited to bleeding, bruising, or infection at the injection site.

Blood draws: Drawing blood may cause slight pain and a small risk of bleeding, bruising, or infection at the injection site.

There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child carried by a woman who takes part in this study. Therefore all women who can become pregnant and are sexually active, or their sexual partners, must use birth control measures while in this study. The following birth control measures are acceptable: diaphragm, birth control pills, injections, intrauterine device (IUD), surgical sterilization, under the skin implants, abstinence. Breast-feeding mothers must stop breast-feeding to take part in this study. Women who can become pregnant must have a pregnancy test before taking part in this study. For the pregnancy test, you will give a blood sample taken from a vein in your arm with a needle within 7days before you enter the study. You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

While you are taking part in this study, you are at risk for the following side effects. You should talk to your study doctor and/or your medical doctor about these side effects. There also may be other side effects that are not known. Side effects may range from mild to life-threatening. Other drugs may be given to lessen side effects. Many side effects go away shortly after the vaccine treatments are stopped, but in some cases side effects can be serious, long lasting, or may never go away. There may be a risk of death.

Are there benefits to taking part in this research study?

This study may not make your health better. However, the information learned may benefit future patients with breast cancer.

What other choices do I have if I don't take part in this research study?

You do not have to be in this study to receive care for your condition. Your other choices may include participation on other clinical studies to no other care at all. You will have regular appointments with your doctor who will check your condition. You should talk to your doctor about your choices before you decide if you will take part in this study.

Will I need to pay for the tests and procedures?

You will not need to pay for any tests and exams that are done just for this research study, including the research blood tests, skin tests and office visits done only for this research study. You will not need to pay for the vaccine used in this study. However, you and/or your health plan will need to pay for all other tests and procedures that are part of this study because they are needed for your regular medical care. You or your health plan might have to pay for other drugs or treatment given to help control side effects. Before you take part in this study, you should call your health insurer to find out if the cost of these tests, procedures, and/or the device will be paid for by the plan. Some health insurers will not pay for these costs. You will have to pay for any costs not covered by your health insurer. If you have questions while at the Clinic, please go to the Admissions and Business Services office, or you may call Patient Account Services at (507) 287-1819.

What happens if I am injured because I took part in this research study?

If you have side effects from the study treatment, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will bill you or your insurer for these services at the usual charge. Mayo will not offer free medical care or payment for any bad side effects from taking part in this study.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, Svetomir N. Markovic, M.D., Ph.D. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

What are my rights if I take part in this research study?

Taking part in this research study does not take away any other rights or benefits you might have if you did not take part in the study. Taking part in this study does not give you any special privileges. You will not be penalized in any way if you decide not to

take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic. If you stop the study you would still receive medical care for your condition although you might not be able to get the study drug.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

Who can answer my questions?

You may talk to Dr. Svetomir N. Markovic at any time about any question you have on this study. You may contact Dr. Markovic (or an associate) by calling the Mayo operator at telephone (507) 284-2511.

You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Marcia Andresen-Reid, the administrator of the Mayo Clinic Office for Human Research Protection, telephone (507) 266-4000 or toll free (866) 273-4681.

Where can I get more information about clinical trials?

You may call the NCI's Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web sites: Cancer Trials: comprehensive clinical trials information
<http://cancertrials.nci.nih.gov>

CancerNetTM: accurate cancer information including PDQ
<http://cancernet.nci.nih.gov>

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic Rochester and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (e.g., date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

This information may be given to other researchers in this study (including those at other institutions), representatives of the sponsor of the study, U. S. Army Medical Research and Material Command, or private, state or federal government parties or regulatory authorities (U.S. and other countries) responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

If this information is given out to anyone outside of Mayo, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information. However, Mayo will take steps to help other parties understand the need to keep this information confidential.

This authorization lasts until the end of the study.

You may stop this authorization at any time by writing to the following address:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

If you do not sign this authorization, or later stop authorization, you may not be able to receive study treatment.

What Other Things Might the Sponsor do with Study Data?

In addition to the uses listed above, companies that sponsor studies often use study data for other purposes that are not part of the study. For example, the company might use the study data for research purposes to support the scientific objectives of the study described in this consent document, to learn more about the effects (good and bad) of any drug,

device or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. Also, the company might share the study data with other companies it does business with. The company might do these things during the study, or after the study has ended, and would not have to ask for your permission to do so. The sponsor might still use study data, even after you stop your authorization, or the authorization expires, as long as the study data was collected before your authorization stopped or expired. The ways in which the study data could be used in the future may not be known now, so we can't give you the details.

A copy of this form will be placed in your medical record.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.

(Date / Time)

(Printed Name of Participant)

(Clinic Number)

(Signature of Participant)

(Date / Time)

(Printed Name of Individual Obtaining Consent)

(Signature of Individual Obtaining Consent)

Consent Form for Participation in a Research Study

TITLE: MC0338, "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas"

IRB #: 782-05 00

RESEARCHER: Dr. E. A. Perez and colleagues

PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD: October 25, 2007

THIS FORM APPROVED: October 25, 2007

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

We are inviting you to participate in this study because of your history of resected breast cancer for which you have already received standard therapy and have no evidence of relapse. Your good overall health and no other history of cancer make it possible for us to offer you participation on this clinical study.

This study is being done to evaluate three different preparations of a breast cancer vaccine to stimulate anti-cancer (T-cell) immune responses and any side effects associated with these vaccinations. The breast cancer vaccine (MUC-1/HER-2/neu peptides) will be combined with one of two immune boosting agents (CpG or GM-CSF) or with immune boosting agents together. These immune boosting agents are believed to be able to make the vaccine more effective. Whether or not this will protect you from breast cancer is still unknown.

The breast cancer vaccine and one of the immune boosting agents (CpG) have not been approved by the Food and Drug Administration (FDA) for commercial use; however, FDA has permitted their use in this research study. GM-CSF is commercially available for use in clinical practice. Laboratory experiments have shown that both of these immune boosting agents are able to make the vaccines more effective in generating an immune response. In laboratory animals, both agents are very effective in boosting anti-

tumor immune responses. However, in clinical trials, neither GM-CSF or CpG have shown effects against cancer, but they both seem to improve the effectiveness of some vaccines. One of the goals of this study is to determine if the unique application of GM-CSF and/or CpG in this study, based on our laboratory data, will make them more likely to improve vaccine immunity.

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If you qualify to take part, you will have blood taken for immunologic testing (to see how your immune system is working) for the study as well as a skin test (allergy test). You will then be randomly assigned (as in the toss of the dice) to be treated with one of three breast cancer vaccines combinations.

Each vaccination will consist of one or two subcutaneous (under the skin) injections of about one half teaspoonful (2ml) of the cancer vaccine and boosting agent combination. The vaccine will be given with Montanide ISA 51, which is an oil that is mixed with the vaccine so that the vaccine can be released into the body slowly. The vaccine will be injected under the skin in areas where there has been no surgery. Usual areas of vaccination include the skin of the upper arms and legs. Vaccinations will be repeated every 4 weeks for 6 months. Before every vaccination, you will be seen by your doctor, examined and 6 tablespoons of blood will be collected to study the development of the anti-cancer immune response. Skin tests will be done prior to vaccinations #6 (see the following table). Evaluations for the cancer (body scans) will be done if and when your doctor thinks it is necessary.

Pre-Study	<ul style="list-style-type: none"> • Routine¹ and research blood tests • Cancer evaluation (scans) • Physical examination • Skin test (Mayo Clinic Rochester, only)
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Vaccination #5 (month 5)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Vaccine treatment
Vaccination #6 (month 6)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment • Skin test (Mayo Clinic Rochester, only)
Every 3 months after the first six months until 5 years after first vaccination	<ul style="list-style-type: none"> • Physical examination • Routine blood tests • Cancer evaluation (scans)
Every 3 months after the first six months until 2 years after first vaccination	<ul style="list-style-type: none"> • Research bloods

1. Routine blood tests include: complete blood count and blood chemistries.

How long will I be in this research study?

You will be on the study for 6 months, and you will be seen in follow-up until 5 years after your first vaccination.

Are there reasons I might leave this research study early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the investigators or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

No. Your samples will be used as described for this study, and then will be destroyed.

What are the risks of this research study?

You will be closely watched by the study team for any side effects. If side effects happen, the study team will take the necessary steps to treat them. This may include stopping the medication and/or stopping the study.

The possible side effects of the following: MUC-1/HER-2/neu, breast cancer vaccine, Montanide ISA 51 (the oil), CpG (immune booster), and GM-CSF (immune booster) include:

Common:

- Injection site reactions: discomfort, rash, redness, firmness, warmth, bleeding, tenderness to touch, numbness, tingling, itching.
- Systemic reactions: skin rash, itching, sweating, muscle aches, joint aches, fatigue.

Rare:

- Injection site reactions: pain, ulceration.
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Although many of these reactions are similar to vaccines that you may have received in the past, the listed side effects that could happen might be more severe. However, we expect that all of these reactions will be very mild. Treatment for these reactions will depend on the type and severity of the reaction. Treatments will be directed at suppressing the immune reaction to the vaccine and may range from mild anti-allergic/anti-inflammatory treatments (for example topical hydrocortisone or Motrin) to more powerful anti-inflammatory therapy including corticosteroids. If severe reactions happen, they may require hospitalization and possibly even minor surgery (severe local skin reactions).

When GM-CSF has been given at higher doses as a daily injection the following side effects have also been reported: diarrhea, general weakness, fever, chills, nausea, vomiting, loss of appetite, headache, pain in the bones, joints and muscles. Most of the symptoms were mild or moderate in severity and were less after taking acetaminophen (Tylenol). Other side effects which happened very rarely were: difficulty breathing, rapid or irregular heart beat or other heart problems, swelling. Even less common, reported

side effects have been the following: 1) increased white cells in the lungs with breathing problems; 2) a syndrome of shortness of breath, low oxygen in the blood, redness in the skin, low blood pressure and dizziness when you stand up or a loss of balance and partial loss of consciousness; 3) serious allergic reactions (like a very severe asthma attack); 4) blood clotting; 5) facial flushing; 6) kidney or liver problems; 7) worsening of fluid accumulation in the arms, legs, lungs or around the heart which may cause problems with breathing or heart failure; 8) patients with heart, lung, kidney or liver problems may have worsening of their symptoms following GM-CSF; and 9) nerve toxicity (weakness, shooting pains, numbness, increased sensitivity to touch, loss of balance, dizziness)

Skin testing: The risks and discomfort of skin testing are minimal and usually limited to bleeding, bruising, or infection at the injection site.

Blood draws: Drawing blood may cause slight pain and a small risk of bleeding, bruising, or infection at the injection site.

There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child carried by a woman who takes part in this study. Therefore all women who can become pregnant and are sexually active, or their sexual partners, must use birth control measures while in this study. The following birth control measures are acceptable: diaphragm, birth control pills, injections, intrauterine device (IUD), surgical sterilization, under the skin implants, abstinence. Breast-feeding mothers must stop breast-feeding to take part in this study. Women who can become pregnant must have a pregnancy test before taking part in this study. For the pregnancy test, you will give a blood sample taken from a vein in your arm with a needle within 7days before you enter the study. You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

While you are taking part in this study, you are at risk for the following side effects. You should talk to your study doctor and/or your medical doctor about these side effects. There also may be other side effects that are not known. Side effects may range from mild to life-threatening. Other drugs may be given to lessen side effects. Many side effects go away shortly after the vaccine treatments are stopped, but in some cases side effects can be serious, long lasting, or may never go away. There may be a risk of death.

Are there benefits to taking part in this research study?

This study may not make your health better. However, the information learned may benefit future patients with breast cancer.

What other choices do I have if I don't take part in this research study?

You do not have to be in this study to receive care for your condition. Your other choices may include participation on other clinical studies to no other care at all. You will have regular appointments with your doctor who will check your condition. You should talk to your doctor about your choices before you decide if you will take part in this study.

Will I need to pay for the tests and procedures?

You will not need to pay for any tests and exams that are done just for this research study, including the research blood tests, skin tests and office visits done only for this research study. You will not need to pay for the vaccine used in this study. However, you and/or your health plan will need to pay for all other tests and procedures that are part of this study because they are needed for your regular medical care. You or your health plan might have to pay for other drugs or treatment given to help control side effects. Before you take part in this study, you should call your health insurer to find out if the cost of these tests, procedures, and/or the device will be paid for by the plan. Some health insurers will not pay for these costs. You will have to pay for any costs not covered by your health insurer. If you have questions while at the Clinic, please go to the Admissions and Business Services office, go to the receptionist at the Registration Desk at the first floor, main lobby of the Davis Building.

What happens if I am injured because I took part in this research study?

If you have side effects from the study treatment, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will bill you or your insurer for these services at the usual charge. Mayo will not offer free medical care or payment for any bad side effects from taking part in this study.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, Svetomir N. Markovic, M.D., Ph.D. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

What are my rights if I take part in this research study?

Taking part in this research study does not take away any other rights or benefits you might have if you did not take part in the study. Taking part in this study does not give you any special privileges. You will not be penalized in any way if you decide not to

take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic. If you stop the study you would still receive medical care for your condition although you might not be able to get the study drug.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

Who can answer my questions?

You may talk to Dr. E. A. Perez at any time about any question you have on this study. You may contact Dr. Perez (or an associate) by calling the Mayo operator at telephone (904) 953-2000.

You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Marcia Andresen-Reid, the administrator of the Mayo Clinic Office for Human Research Protection, telephone (507) 266-4000 or toll free (866) 273-4681.

Where can I get more information about clinical trials?

You may call the NCI's Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web sites: Cancer Trials: comprehensive clinical trials information
<http://cancertrials.nci.nih.gov>

CancerNet™: accurate cancer information including PDQ
<http://cancernet.nci.nih.gov>

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic Jacksonville and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (e.g., date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

This information may be given to other researchers in this study (including those at other institutions), representatives of the sponsor of the study, U. S. Army Medical Research and Material Command, or private, state or federal government parties or regulatory authorities (U.S. and other countries) responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

If this information is given out to anyone outside of Mayo, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information. However, Mayo will take steps to help other parties understand the need to keep this information confidential.

This authorization lasts until the end of the study.

You may stop this authorization at any time by writing to the following address:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

If you do not sign this authorization, or later stop authorization, you may not be able to receive study treatment.

What Other Things Might the Sponsor do with Study Data?

In addition to the uses listed above, companies that sponsor studies often use study data for other purposes that are not part of the study. For example, the company might use the study data for research purposes to support the scientific objectives of the study described in this consent document, to learn more about the effects (good and bad) of any drug,

device or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. Also, the company might share the study data with other companies it does business with. The company might do these things during the study, or after the study has ended, and would not have to ask for your permission to do so. The sponsor might still use study data, even after you stop your authorization, or the authorization expires, as long as the study data was collected before your authorization stopped or expired. The ways in which the study data could be used in the future may not be known now, so we can't give you the details.

A copy of this form will be placed in your medical record.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.

(Date / Time)

(Printed Name of Participant)

(Clinic Number)

(Signature of Participant)

(Date / Time)

(Printed Name of Individual Obtaining Consent)

(Signature of Individual Obtaining Consent)

(Date / Time)

(Printed Name of Witness Signature)

(Signed Name of Witness Signature)

Consent Form for Participation in a Research Study

TITLE: MC0338, "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas"

IRB #: 782-05 00

RESEARCHER: Dr. B. A. Pockaj and colleagues

PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD: October 25, 2007

THIS FORM APPROVED: October 25, 2007

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

We are inviting you to participate in this study because of your history of resected breast cancer for which you have already received standard therapy and have no evidence of relapse. Your good overall health and no other history of cancer make it possible for us to offer you participation on this clinical study.

This study is being done to evaluate three different preparations of a breast cancer vaccine to stimulate anti-cancer (T-cell) immune responses and any side effects associated with these vaccinations. The breast cancer vaccine (MUC-1/HER-2/neu peptides) will be combined with one of two immune boosting agents (CpG or GM-CSF) or with immune boosting agents together. These immune boosting agents are believed to be able to make the vaccine more effective. Whether or not this will protect you from breast cancer is still unknown.

The breast cancer vaccine and one of the immune boosting agents (CpG) have not been approved by the Food and Drug Administration (FDA) for commercial use; however, FDA has permitted their use in this research study. GM-CSF is commercially available for use in clinical practice. Laboratory experiments have shown that both of these immune boosting agents are able to make the vaccines more effective in generating an immune response. In laboratory animals, both agents are very effective in boosting anti-

tumor immune responses. However, in clinical trials, neither GM-CSF or CpG have shown effects against cancer, but they both seem to improve the effectiveness of some vaccines. One of the goals of this study is to determine if the unique application of GM-CSF and/or CpG in this study, based on our laboratory data, will make them more likely to improve vaccine immunity.

This study is sponsored by the Department of Defense.

How many people will take part in this research study?

The plan is to have up to 45 people take part in this study at Mayo Clinic Rochester, Jacksonville, and Arizona. Up to 60 people may be screened to find enough eligible people to begin the study.

What will happen in this research study?

Before you enter the study, you will have a physical examination and blood tests to make sure that you qualify to take part in this study. About 6 tablespoons of blood will be taken for testing.

If you qualify to take part, you will have blood taken for immunologic testing (to see how your immune system is working) for the study as well as a skin test (allergy test). You will then be randomly assigned (as in the toss of the dice) to be treated with one of three breast cancer vaccines combinations.

Each vaccination will consist of one or two subcutaneous (under the skin) injections of about one half teaspoonful (2ml) of the cancer vaccine and boosting agent combination. The vaccine will be given with Montanide ISA 51, which is an oil that is mixed with the vaccine so that the vaccine can be released into the body slowly. The vaccine will be injected under the skin in areas where there has been no surgery. Usual areas of vaccination include the skin of the upper arms and legs. Vaccinations will be repeated every 4 weeks for 6 months. Before every vaccination, you will be seen by your doctor, examined and 6 tablespoons of blood will be collected to study the development of the anti-cancer immune response. Skin tests will be done prior to vaccinations #6 (see the following table). Evaluations for the cancer (body scans) will be done if and when your doctor thinks it is necessary.

Pre-Study	<ul style="list-style-type: none"> • Routine¹ and research blood tests • Cancer evaluation (scans) • Physical examination • Skin test (Mayo Clinic Rochester, only)
Vaccination #1 (month 1)	<ul style="list-style-type: none"> • Vaccine treatment
Vaccination #2 (month 2)	<ul style="list-style-type: none"> • Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #3 (month 3)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment
Vaccination #4 (month 4)	<ul style="list-style-type: none"> • Routine blood test collection • Physical examination • Vaccine treatment
Vaccination #5 (month 5)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Vaccine treatment
Vaccination #6 (month 6)	<ul style="list-style-type: none"> • Routine and research blood test collection • Physical examination • Cancer evaluation (scans) • Vaccine treatment • Skin test (Mayo Clinic Rochester, only)
Every 3 months after the first six months until 5 years after first vaccination	<ul style="list-style-type: none"> • Physical examination • Routine blood tests • Cancer evaluation (scans)
Every 3 months after the first six months until 2 years after first vaccination	<ul style="list-style-type: none"> • Research bloods

1. Routine blood tests include: complete blood count and blood chemistries.

How long will I be in this research study?

You will be on the study for 6 months, and you will be seen in follow-up until 5 years after your first vaccination.

Are there reasons I might leave this research study early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the investigators or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

No. Your samples will be used as described for this study, and then will be destroyed.

What are the risks of this research study?

You will be closely watched by the study team for any side effects. If side effects happen, the study team will take the necessary steps to treat them. This may include stopping the medication and/or stopping the study.

The possible side effects of the following: MUC-1/HER-2/neu, breast cancer vaccine, Montanide ISA 51 (the oil), CpG (immune booster), and GM-CSF (immune booster) include:

Common:

- Injection site reactions: discomfort, rash, redness, firmness, warmth, bleeding, tenderness to touch, numbness, tingling, itching.
- Systemic reactions: skin rash, itching, sweating, muscle aches, joint aches, fatigue.

Rare:

- Injection site reactions: pain, ulceration.
- Systemic reactions: low blood counts, difficulty breathing, heart problems, blood clots, seizures, infection, kidney and liver problems, headache, stomach pain, cough.

Although many of these reactions are similar to vaccines that you may have received in the past, the listed side effects that could happen might be more severe. However, we expect that all of these reactions will be very mild. Treatment for these reactions will depend on the type and severity of the reaction. Treatments will be directed at suppressing the immune reaction to the vaccine and may range from mild anti-allergic/anti-inflammatory treatments (for example topical hydrocortisone or Motrin) to more powerful anti-inflammatory therapy including corticosteroids. If severe reactions happen, they may require hospitalization and possibly even minor surgery (severe local skin reactions).

When GM-CSF has been given at higher doses as a daily injection the following side effects have also been reported: diarrhea, general weakness, fever, chills, nausea, vomiting, loss of appetite, headache, pain in the bones, joints and muscles. Most of the symptoms were mild or moderate in severity and were less after taking acetaminophen (Tylenol). Other side effects which happened very rarely were: difficulty breathing, rapid or irregular heart beat or other heart problems, swelling. Even less common, reported

side effects have been the following: 1) increased white cells in the lungs with breathing problems; 2) a syndrome of shortness of breath, low oxygen in the blood, redness in the skin, low blood pressure and dizziness when you stand up or a loss of balance and partial loss of consciousness; 3) serious allergic reactions (like a very severe asthma attack); 4) blood clotting; 5) facial flushing; 6) kidney or liver problems; 7) worsening of fluid accumulation in the arms, legs, lungs or around the heart which may cause problems with breathing or heart failure; 8) patients with heart, lung, kidney or liver problems may have worsening of their symptoms following GM-CSF; and 9) nerve toxicity (weakness, shooting pains, numbness, increased sensitivity to touch, loss of balance, dizziness)

Skin testing: The risks and discomfort of skin testing are minimal and usually limited to bleeding, bruising, or infection at the injection site.

Blood draws: Drawing blood may cause slight pain and a small risk of bleeding, bruising, or infection at the injection site.

There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child carried by a woman who takes part in this study. Therefore all women who can become pregnant and are sexually active, or their sexual partners, must use birth control measures while in this study. The following birth control measures are acceptable: diaphragm, birth control pills, injections, intrauterine device (IUD), surgical sterilization, under the skin implants, abstinence. Breast-feeding mothers must stop breast-feeding to take part in this study. Women who can become pregnant must have a pregnancy test before taking part in this study. For the pregnancy test, you will give a blood sample taken from a vein in your arm with a needle within 7days before you enter the study. You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

While you are taking part in this study, you are at risk for the following side effects. You should talk to your study doctor and/or your medical doctor about these side effects. There also may be other side effects that are not known. Side effects may range from mild to life-threatening. Other drugs may be given to lessen side effects. Many side effects go away shortly after the vaccine treatments are stopped, but in some cases side effects can be serious, long lasting, or may never go away. There may be a risk of death.

Are there benefits to taking part in this research study?

This study may not make your health better. However, the information learned may benefit future patients with breast cancer.

What other choices do I have if I don't take part in this research study?

You do not have to be in this study to receive care for your condition. Your other choices may include participation on other clinical studies to no other care at all. You will have regular appointments with your doctor who will check your condition. You should talk to your doctor about your choices before you decide if you will take part in this study.

Will I need to pay for the tests and procedures?

You will not need to pay for any tests and exams that are done just for this research study, including the research blood tests, skin tests and office visits done only for this research study. You will not need to pay for the vaccine used in this study. However, you and/or your health plan will need to pay for all other tests and procedures that are part of this study because they are needed for your regular medical care. You or your health plan might have to pay for other drugs or treatment given to help control side effects. Before you take part in this study, you should call your health insurer to find out if the cost of these tests, procedures, and/or the device will be paid for by the plan. Some health insurers will not pay for these costs. You will have to pay for any costs not covered by your health insurer. If you have questions while at the Clinic, please go to the Admissions and Business Services office, please go to the concourse level Patient Financial Services office or call this office at (480) 301-8000.

What happens if I am injured because I took part in this research study?

If you have side effects from the study treatment, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will bill you or your insurer for these services at the usual charge. Mayo will not offer free medical care or payment for any bad side effects from taking part in this study.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, Svetomir N. Markovic, M.D., Ph.D. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

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take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic. If you stop the study you would still receive medical care for your condition although you might not be able to get the study drug.

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You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Marcia Andresen-Reid, the administrator of the Mayo Clinic Office for Human Research Protection, telephone (507) 266-4000 or toll free (866) 273-4681.

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This information may be given to other researchers in this study (including those at other institutions), representatives of the sponsor of the study, U. S. Army Medical Research and Material Command, or private, state or federal government parties or regulatory authorities (U.S. and other countries) responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

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You may stop this authorization at any time by writing to the following address:

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200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

If you do not sign this authorization, or later stop authorization, you may not be able to receive study treatment.

What Other Things Might the Sponsor do with Study Data?

In addition to the uses listed above, companies that sponsor studies often use study data for other purposes that are not part of the study. For example, the company might use the study data for research purposes to support the scientific objectives of the study described in this consent document, to learn more about the effects (good and bad) of any drug,

device or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. Also, the company might share the study data with other companies it does business with. The company might do these things during the study, or after the study has ended, and would not have to ask for your permission to do so. The sponsor might still use study data, even after you stop your authorization, or the authorization expires, as long as the study data was collected before your authorization stopped or expired. The ways in which the study data could be used in the future may not be known now, so we can't give you the details.

A copy of this form will be placed in your medical record.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.

(Date / Time)

(Printed Name of Participant)

(Clinic Number)

(Signature of Participant)

(Date / Time)

(Printed Name of Individual Obtaining Consent)

(Signature of Individual Obtaining Consent)

-----Original Message-----

From: Duchesneau, Caryn L Ms USAMRMC [mailto:Caryn.Duchesneau@us.army.mil]
Sent: Monday, June 09, 2008 2:04 PM
To: Eichelberger, Brian J Mr USAMRAA
Cc: Bennett, Jodi H Ms USAMRMC; Gendler, Sandra J., Ph.D.; Wilberding, Julie A Dr AMDEX; Dolney, Suzanne E Ms AMDEX; Moore, Katherine H Dr USAMRMC; Brosch, Laura R COL USAMRMC; Duchesneau, Caryn L Ms USAMRMC; Markovic, Svetomir N., M.D., Ph.D.; Dustin, Kelly Ms AMDEX
Subject: A-10856, Amendment Approval Memorandum (Proposal Log Number BC004043, Award Number DAMD17-01-1-0318) (UNCLASSIFIED)

Classification: UNCLASSIFIED

Caveats: NONE

SUBJECT: Amendment for the Protocol, "MUC1/HER-2/neu Peptide Based Immunotherapeutic Vaccines for Breast Adenocarcinomas," Submitted by Svetomir N. Markovic, MD, Mayo Clinic, Rochester Minnesota, in Support of the Proposal, "Immunotherapeutic Strategies in Breast Cancer: Preclinical and Clinical Trials," Submitted by Sandra J. Gendler, PhD, Mayo Clinic, Scottsdale, Arizona, Proposal Log Number BC004043, Award Number DAMD17-01-1-0318, HRPO Log Number A-10856

1. The subject protocol received final approval by the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) on 29 January 2007.
2. An amendment to this greater than minimal risk protocol was received by the HRPO on 19 May 2008. The amendment was approved by the Mayo Clinic Institutional Review Board on 29 May 2008.
3. The amendment allows the following protocol revisions: (1) Clarification of MUC1 positive breast cancer confirmation by central review prior to randomization; (2) Addition to the inclusion criteria that women of childbearing potential should have a negative serum pregnancy test done ??7 days before registration; (3) Clarification to the timing of research blood draws; (4) Removal of Kathleen Liffrig from the study; and (5) Other administrative and editorial changes.
4. The changes proposed in the amendment have been reviewed by the HRPO and found to be acceptable. The protocol amendment is approved.
5. The Principal Investigator remains responsible for fulfilling reporting requirements to the HRPO as outlined in the initial approval memo dated 29 January 2007.
6. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project
7. The HRPO point of contact for this action is Suzanne E. Dolney, BSN, RN, Human Subjects Protection Scientist, at extension 301-619-6657/suzanne.dolney@us.army.mil. The point of contact for this action is Kelly Dustin, RN, MS, CCRC, at 301-619-2380/kelly.dustin@us.army.mil.

CARYN L. DUCHESNEAU, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
U.S. Army Medical Research and Materiel Command

NOTE: The official signed copy of this approval memo is housed with the protocol file at the Office of Research Protections, 504 Scott Street, Fort Detrick, MD 21702. Signed copies will be provided upon request.

Classification: UNCLASSIFIED

Caveats: NONE